

CONFIDENTIAL

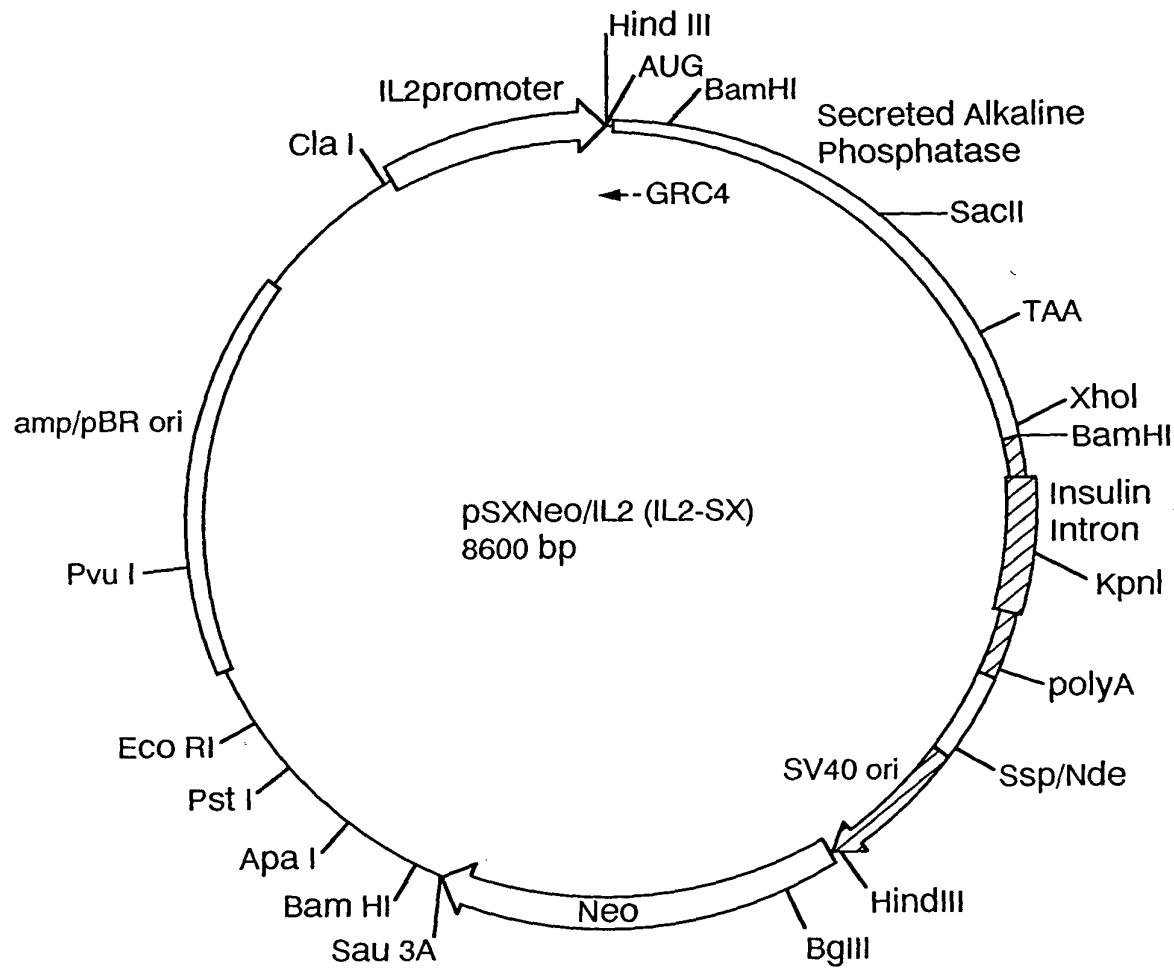
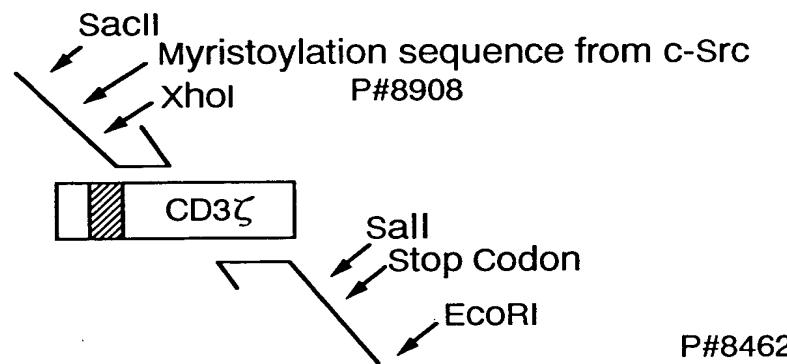


FIG. 1

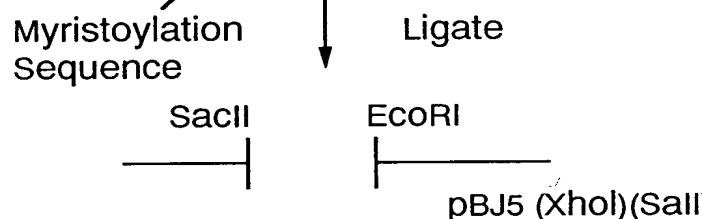
Construction of intracellular signalling chimera:

1. PCR myristoylated CD3 $\zeta$



SacII/Xhol      Sall/EcoRI

2. Cut and clone PCR fragment



\*The MZE series contains a 9aa HA epitope at the 3' end.

3. SEQUENCE insert

From plasmid #FK12/KS

4. Cut at Xhol or Sall and add FKBP domains

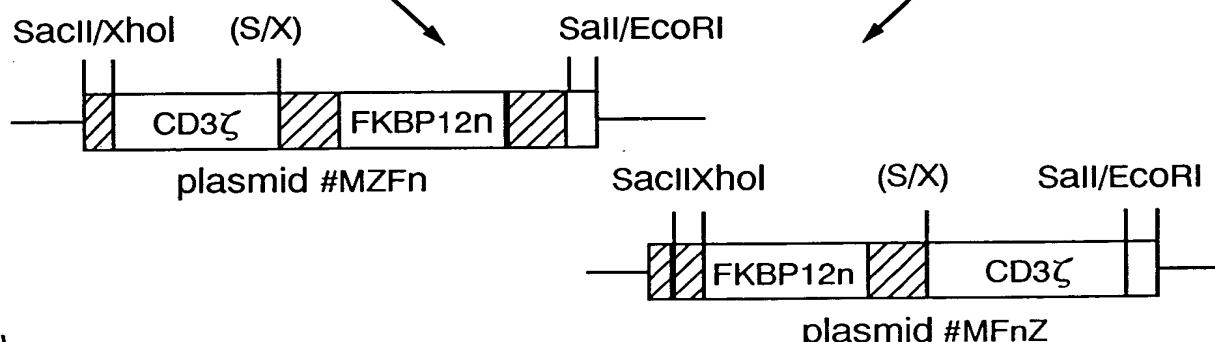
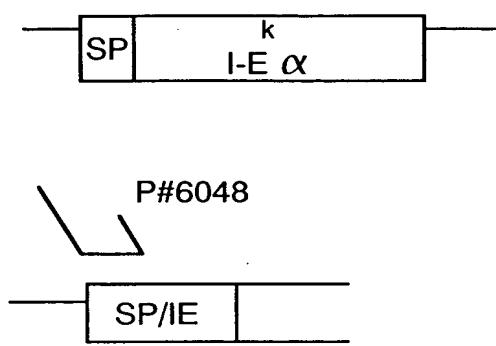


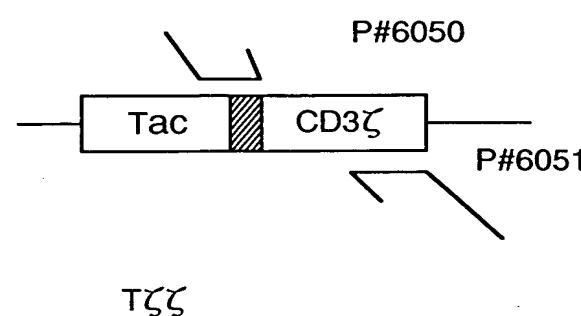
FIG. 2

Construction of extracellular signaling chimera:

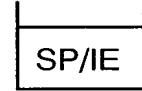
1. PCT murine signal peptide



2. PCT CD3 trans-membrane and cytoplasmic domains

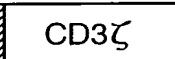


SacII Xhol



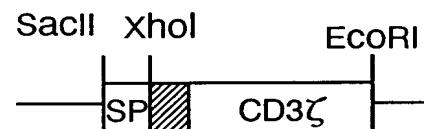
Xhol

EcoRI



SacII EcoRI

pBluescript

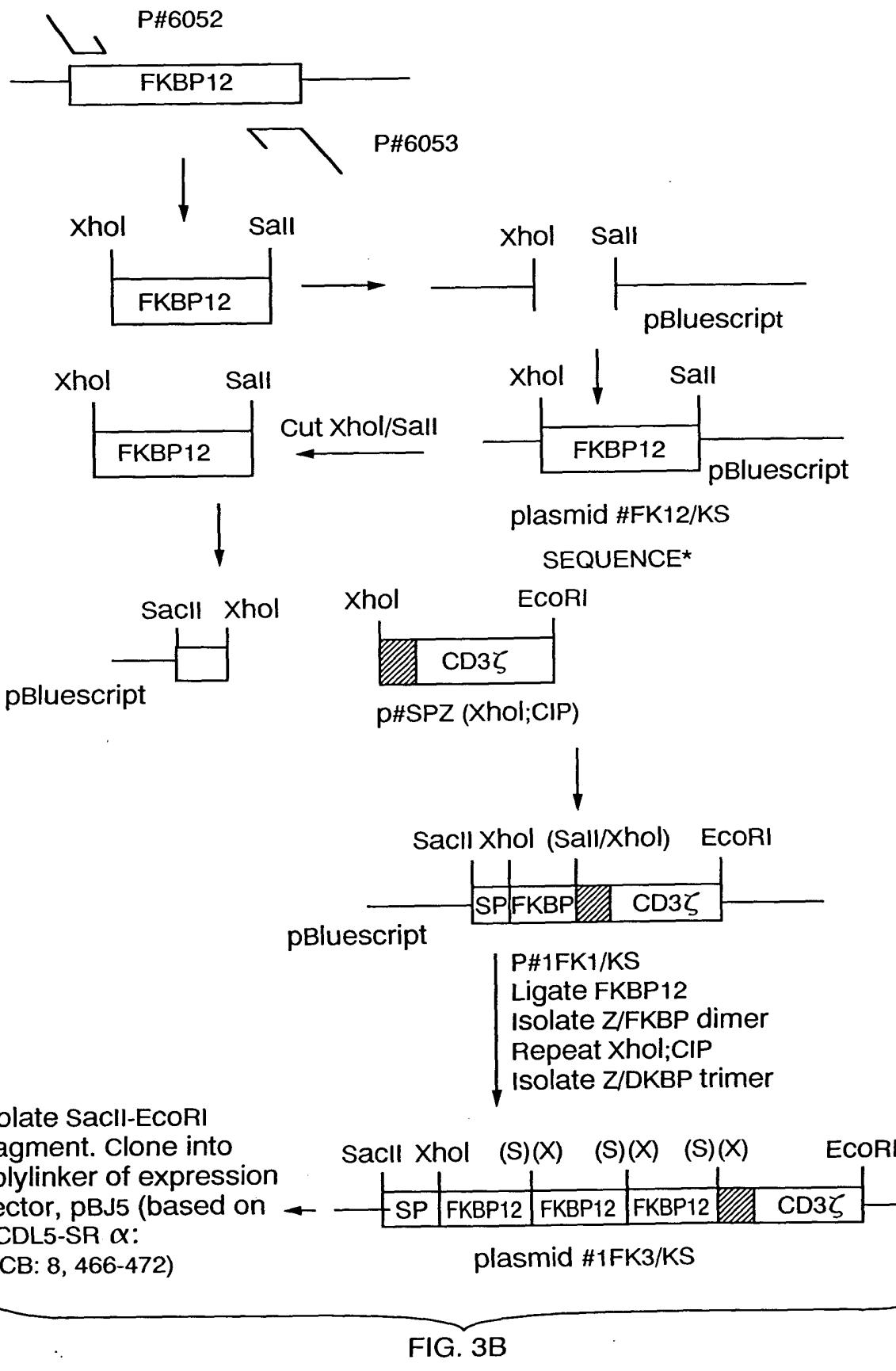


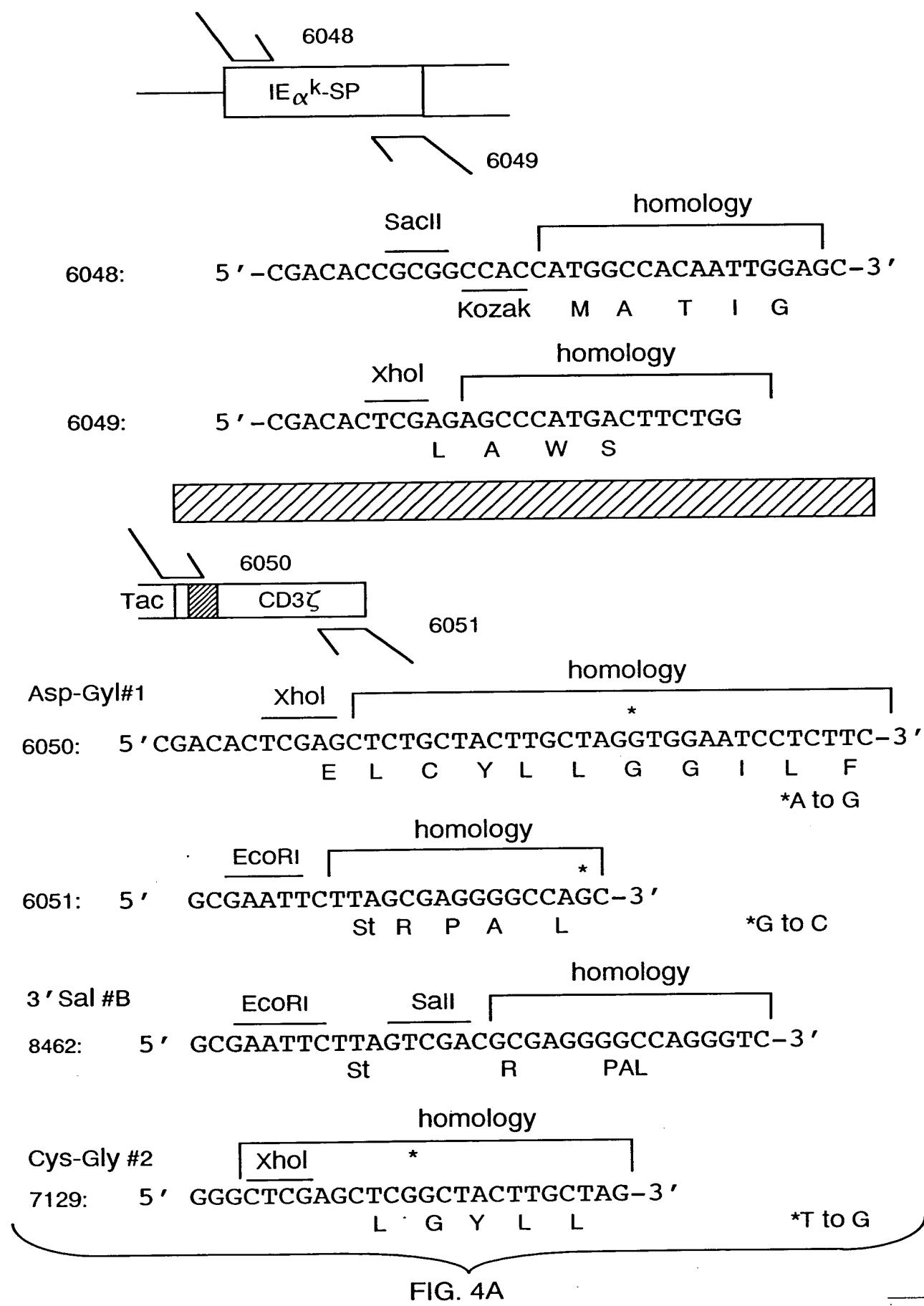
plasmid #SPZ/KS  
 SEQUENCE insert\*

Cut Xhol

FIG. 3A

### 3. PCR FKBP12





CYCC

6568: Xhol homology  
5' -CGACACTCGAGGTGACGGACAAGGTC-3'  
6569: Sall homology  
5' -CGACAGTCGACCCAATCAGGGACCTC-3'

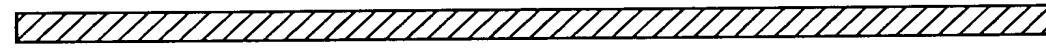


EPITOPE

7850: Xhol BsiWI  
5' -TCGAGTATCCGTACGACGTACCAAGACTACGCAG-3'  
Y P Y D V P D Y A  
7851: Sall  
5' -TCGACTGCGTAGTCTGGTACGTCGTACGGATAC-3'

EPITOPE: 5SEP, 3XEP

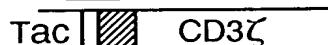
8922: Sall  
5' -TCGACTATCCGTACGACGTACCAAGACTACGCAC-3'  
8923: Xhol  
5' -TCGAGTGCCTAGTCTGGTACGTCGTACGGATAG-3'



Myristoylation from c-src 5SMXZ

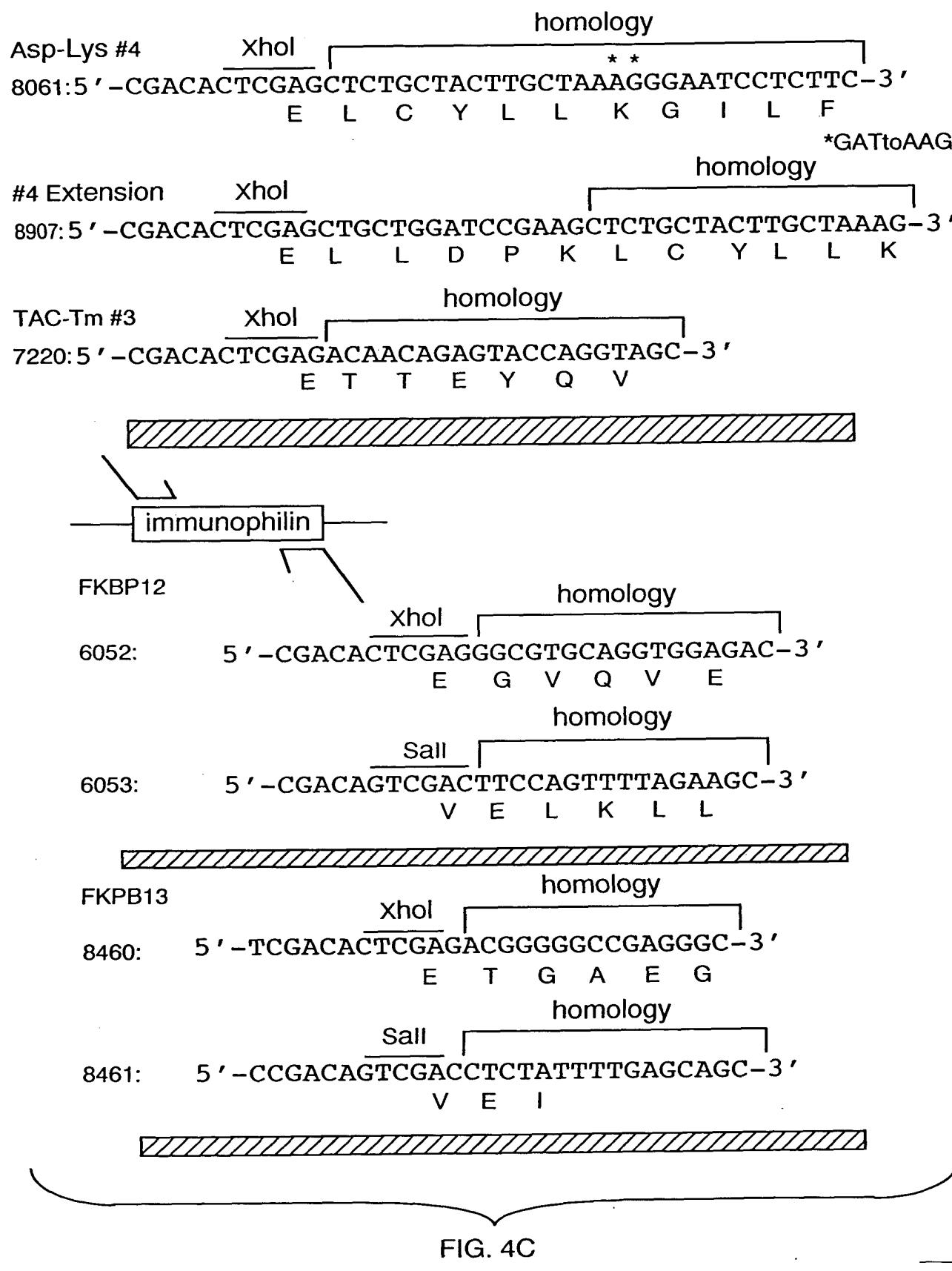
8908: SacII  
5' -CGACACCGCGGCCACCATGGGGAGTAGCAAGAGCAAGCCT  
KOZAK M G S S K S K P  
AAGGACCCCCAGCCAGCGCCCTCGAGAGGAGTGCAGAGACTG-3'  
K D P S Q R L E R S A E T



5XTZ  
Tac 

8912: Xhol homology  
5' -CGACACTCGAGGGAGCTCTGTGACGATG-3'  
E L C D D

FIG. 4B



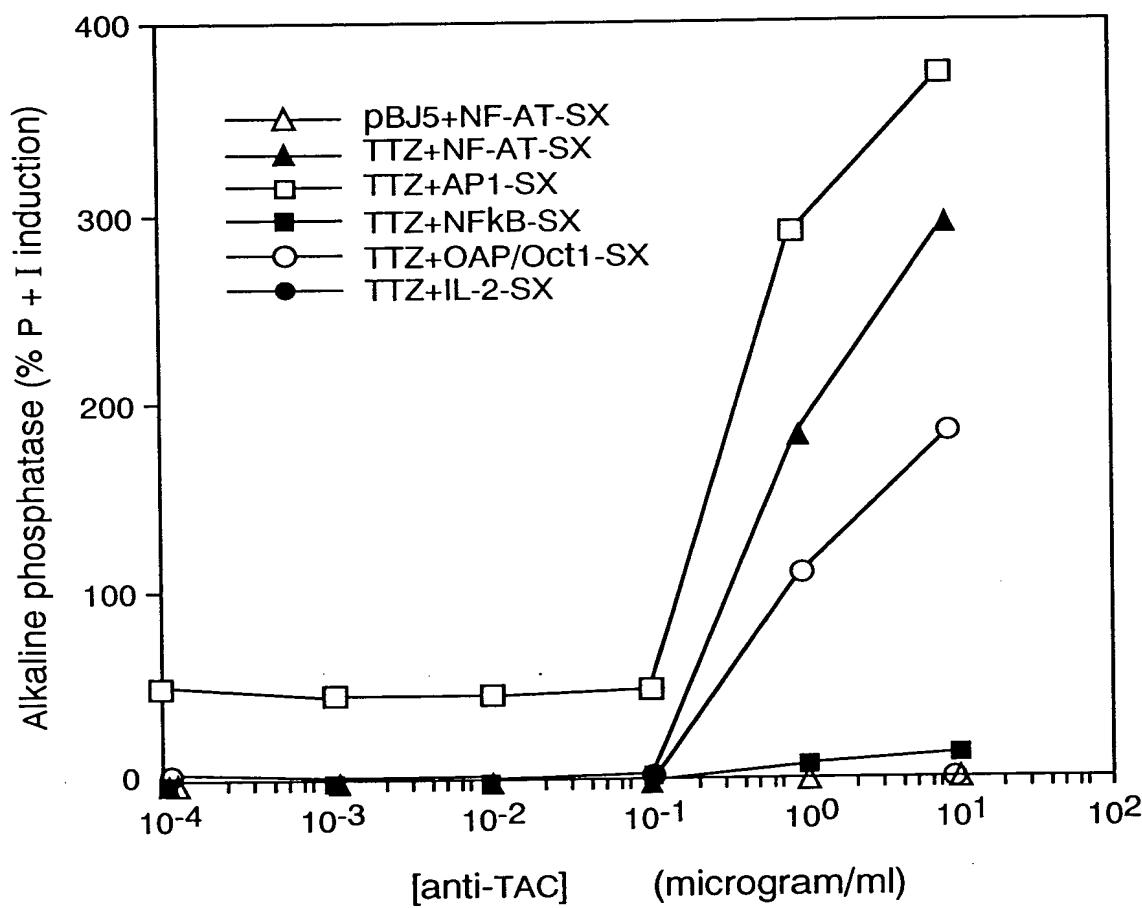


FIG. 5

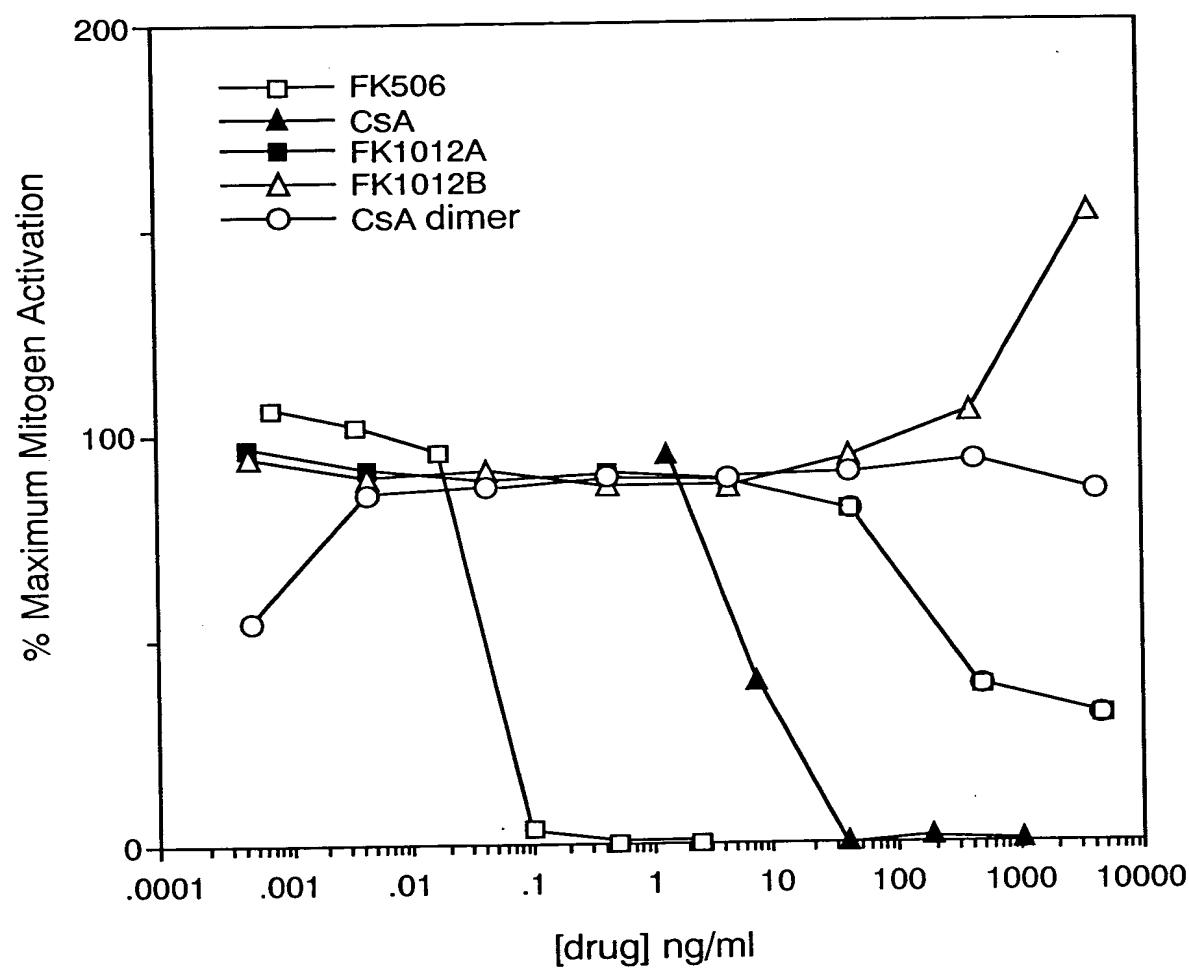
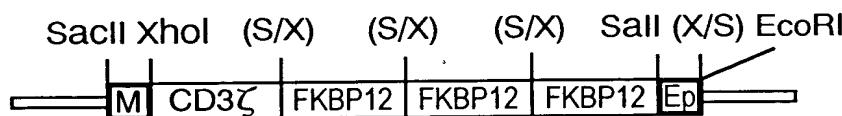
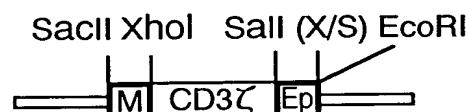


FIG. 6A

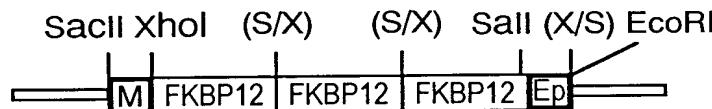


MZF3E

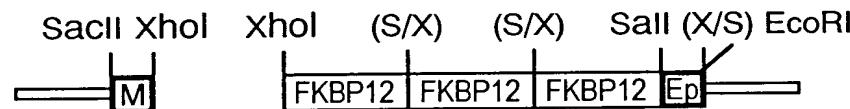


MZE

Cut Xhol/Sall; CIP; + FKBP12X3



MF3E



1



1. Cytoplasmic moiety of surface receptor
2. Tyrosine Kinase
3. Transcription Factor
4. Others

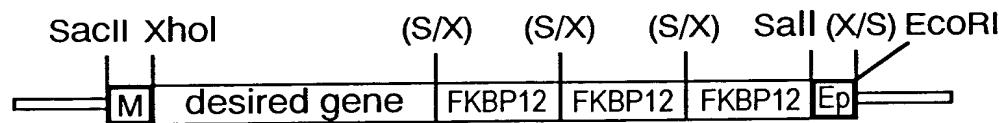


FIG. 6B

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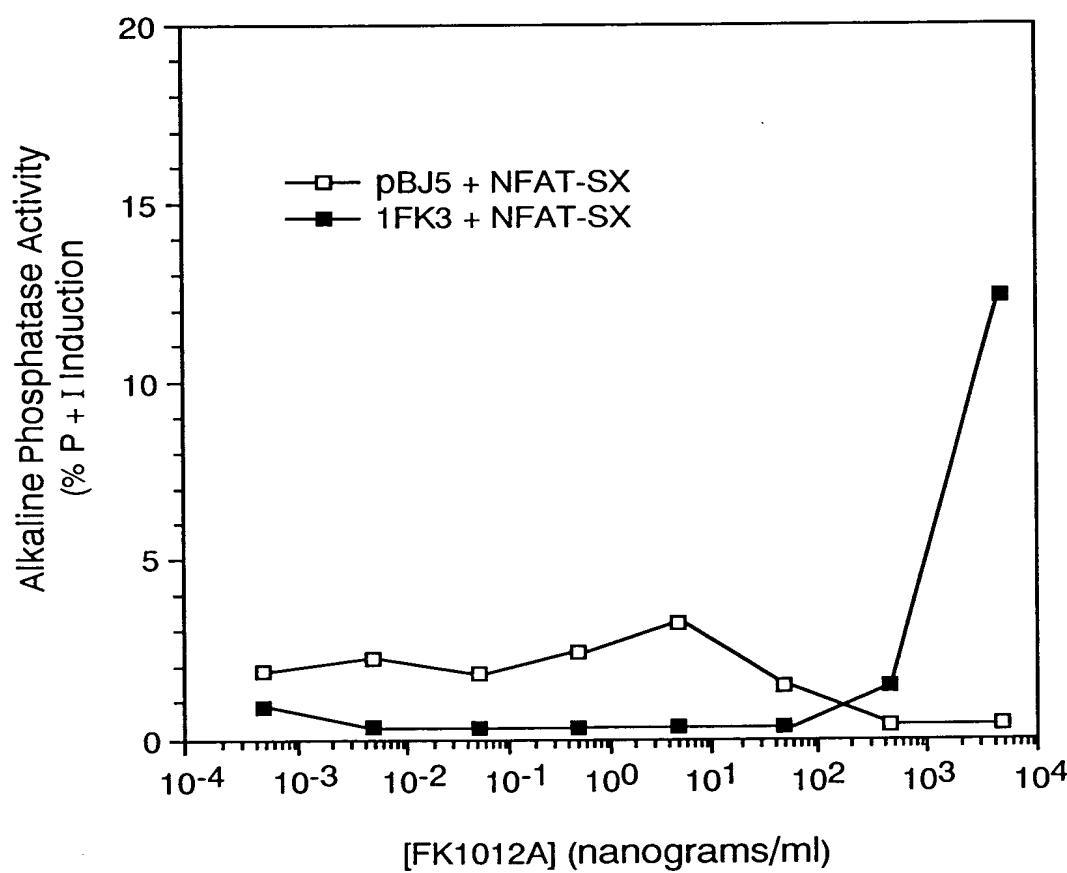


FIG. 7

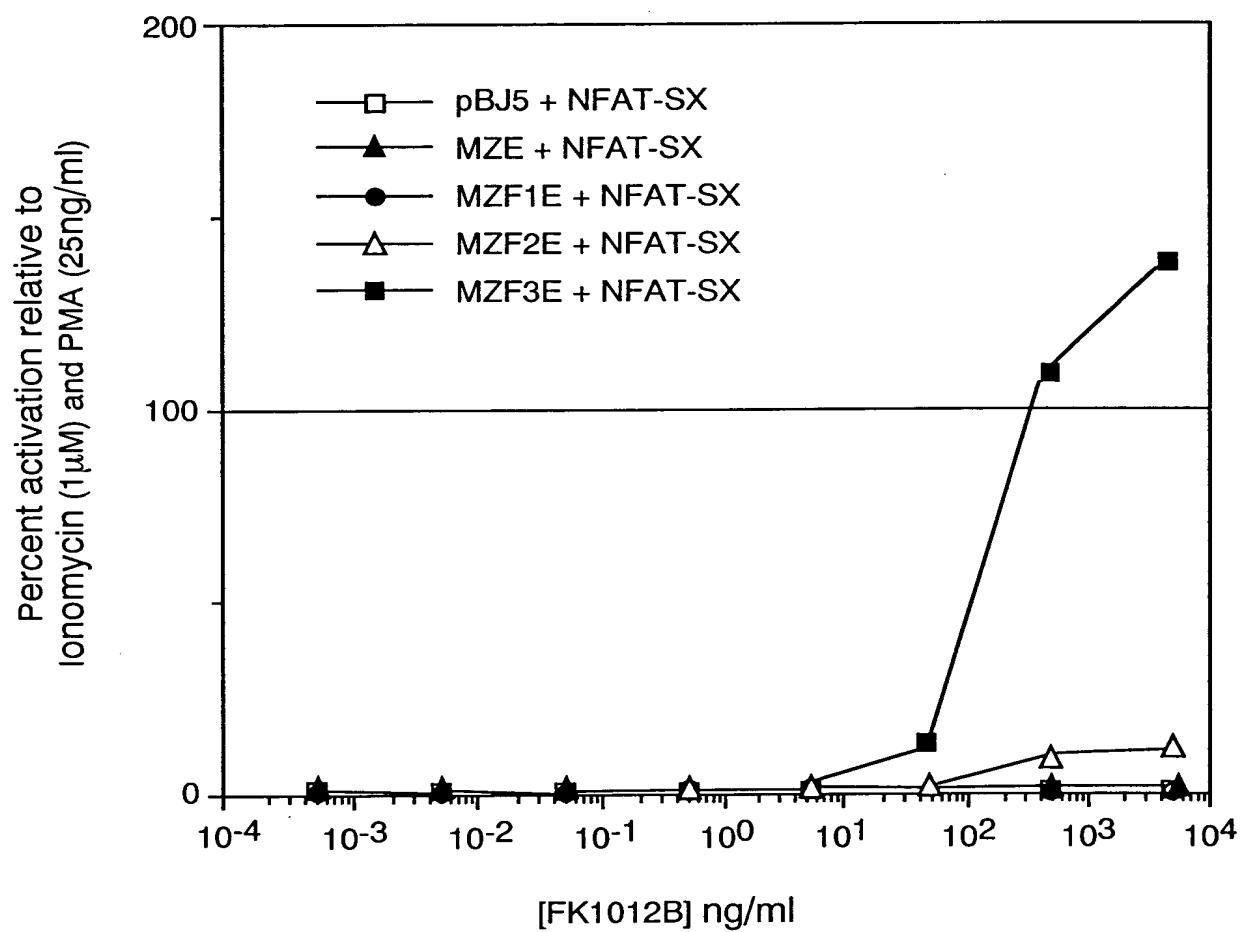


FIG. 8

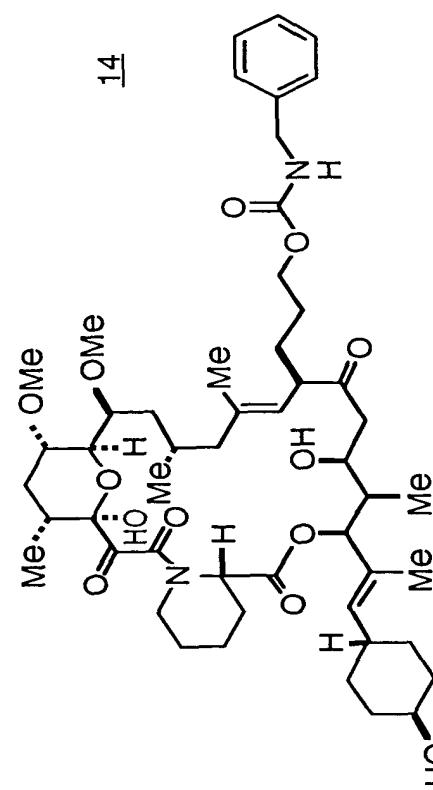
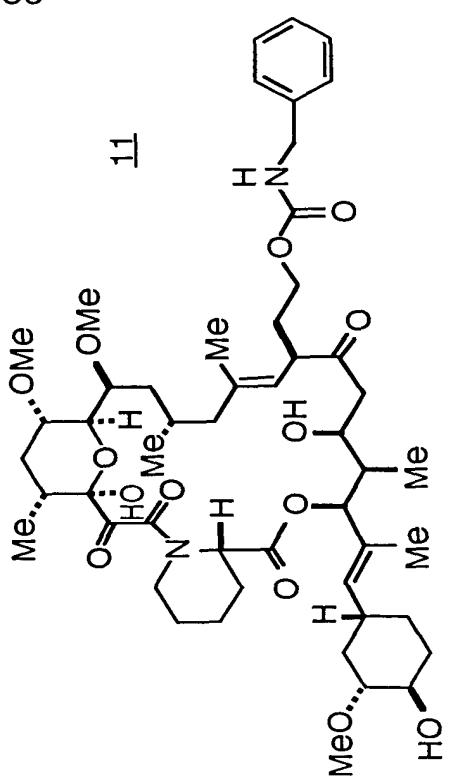
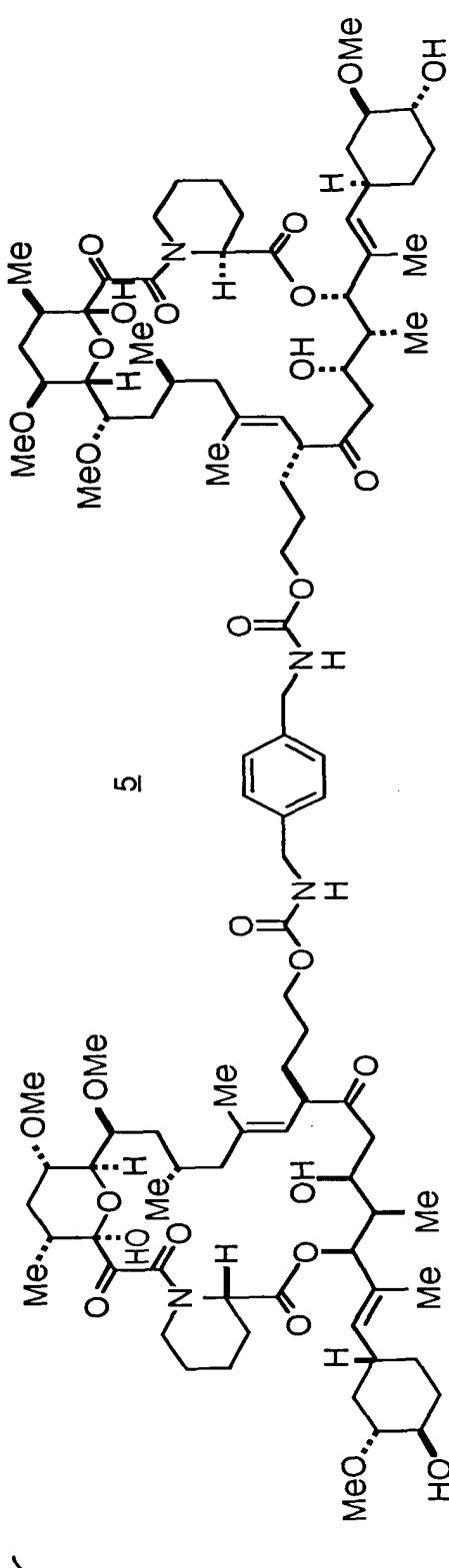
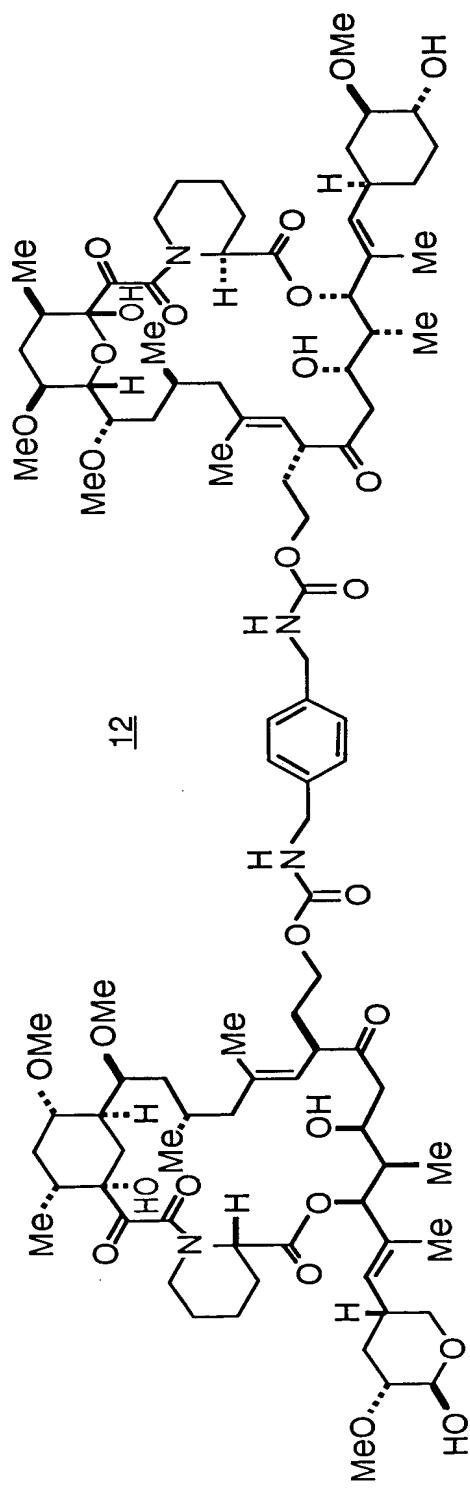
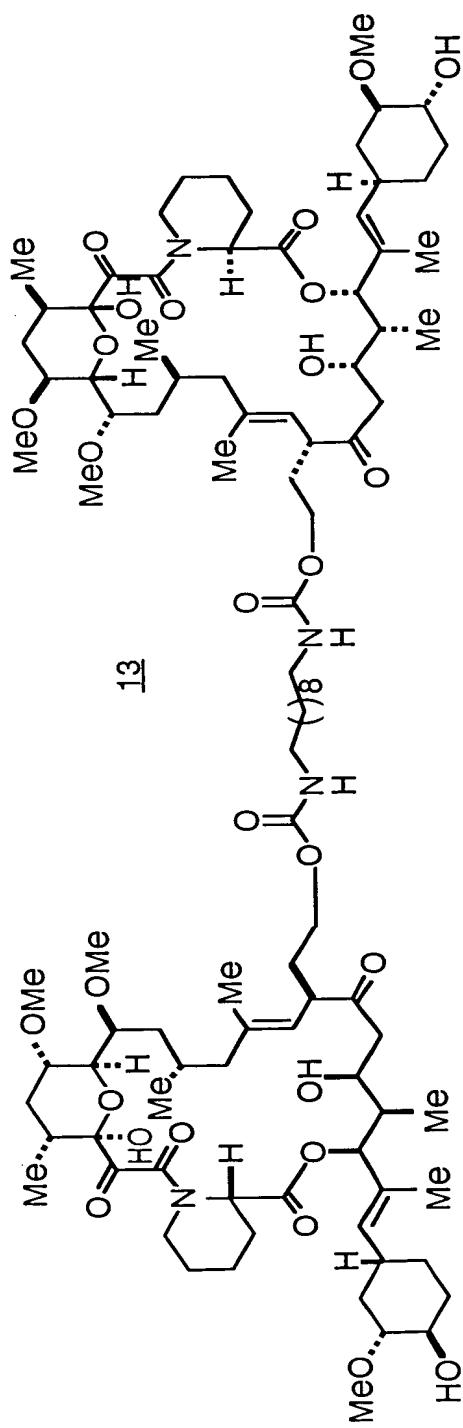


FIG. 9A

FIG. 9B



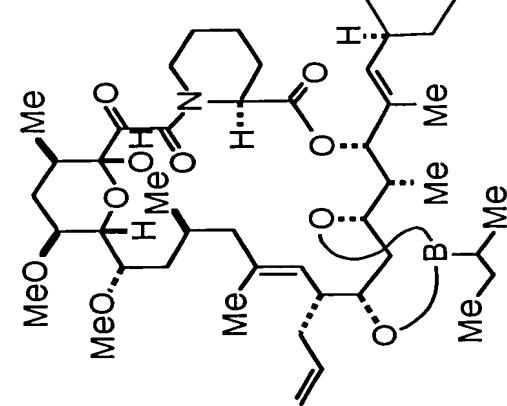
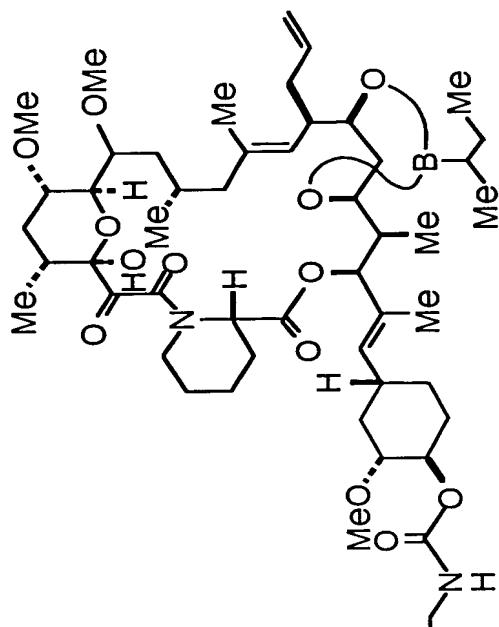
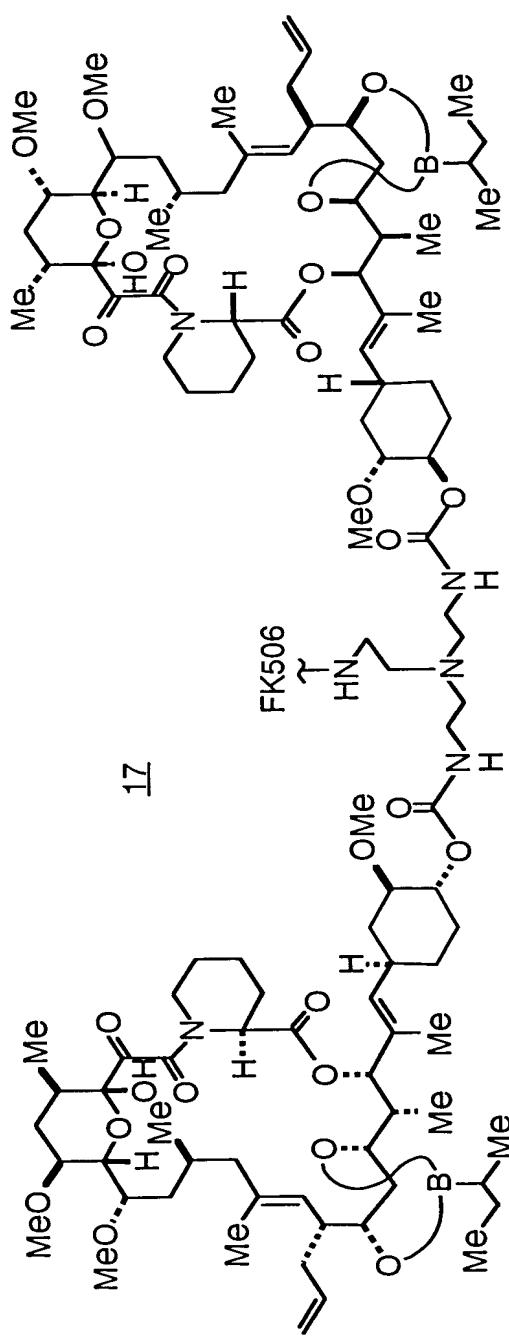
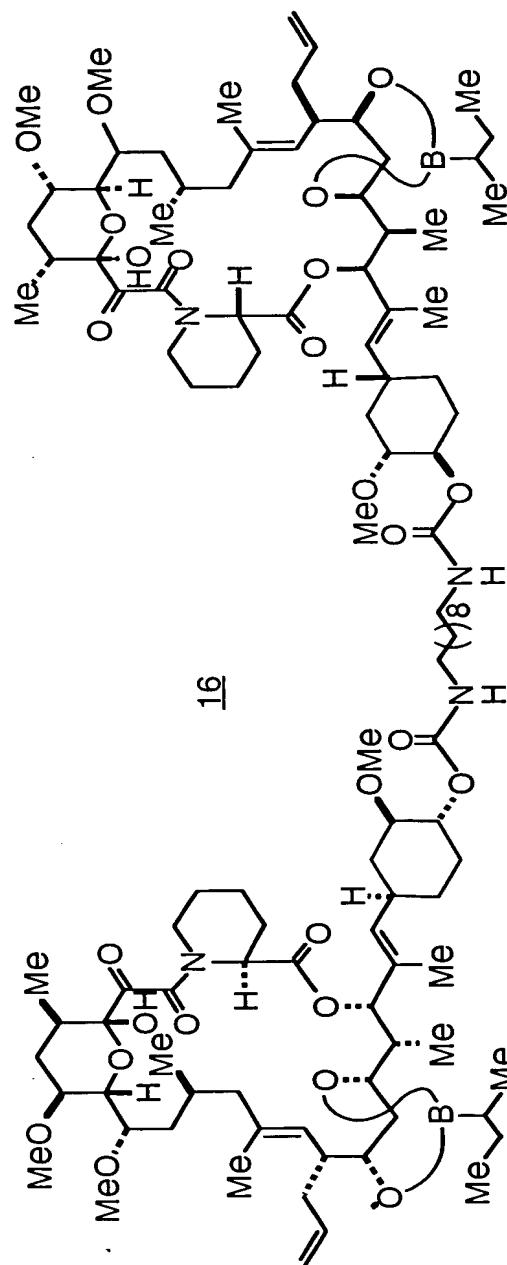
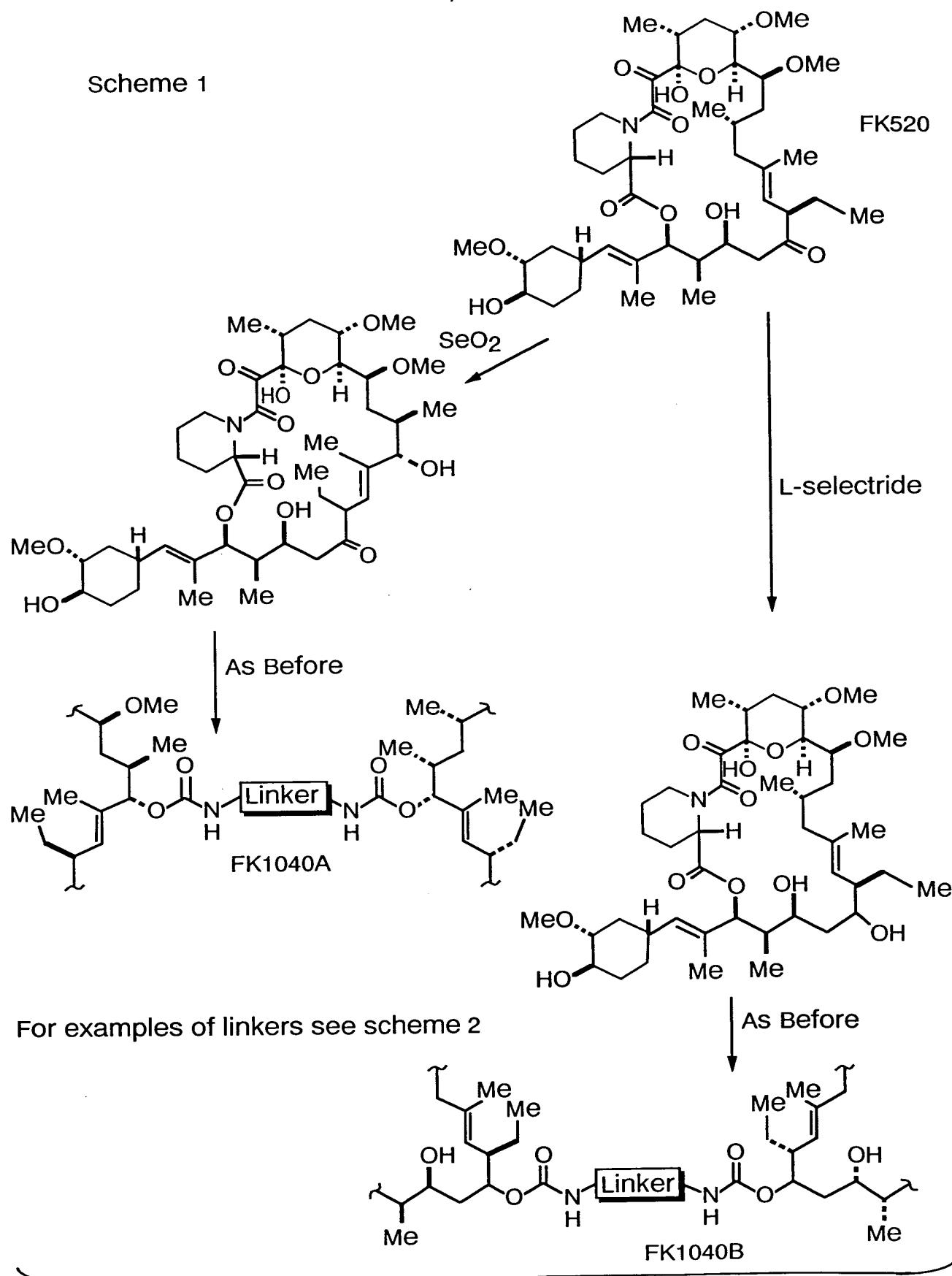


FIG. 9C

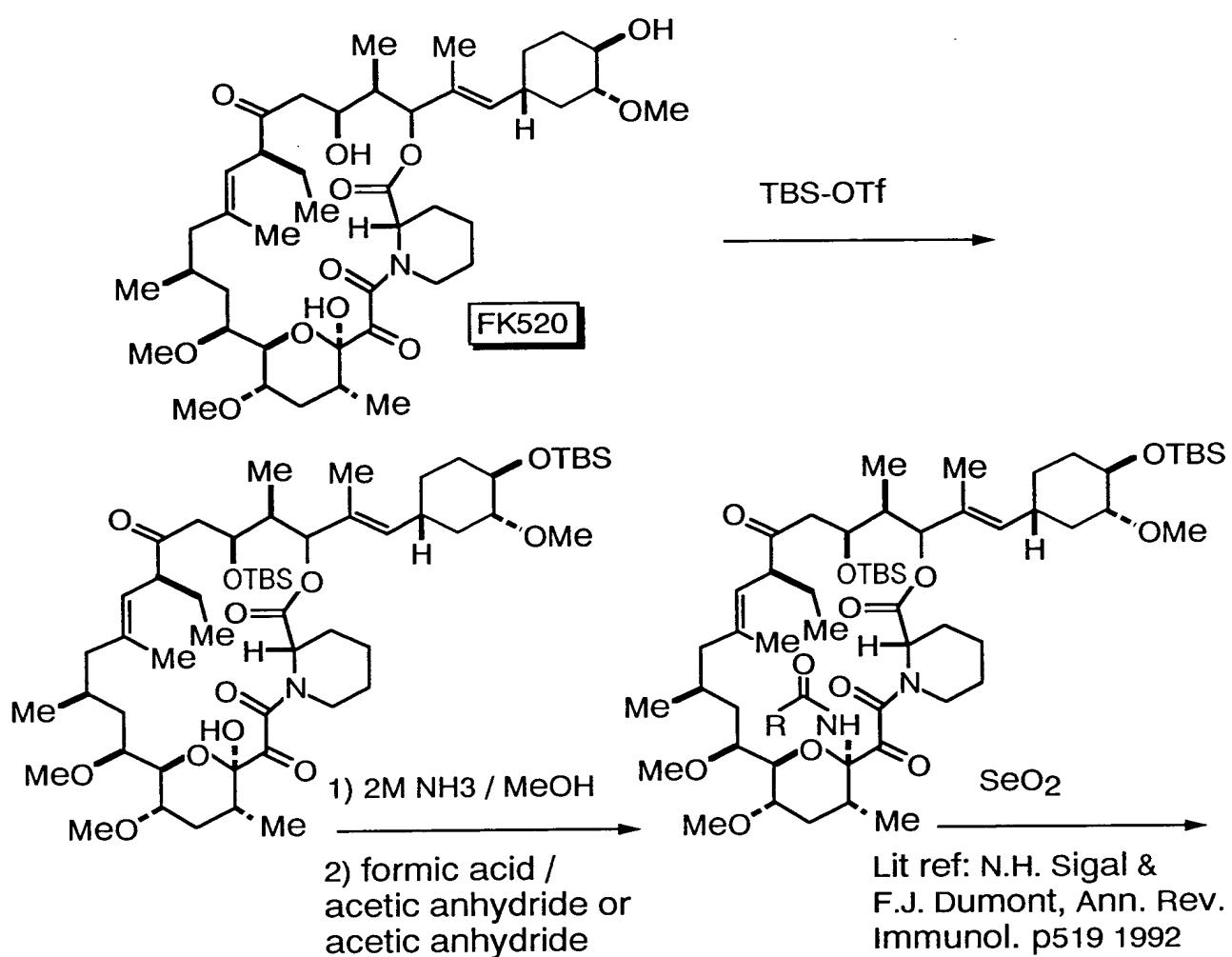
FIG. 9D



Scheme 1



Scheme 2: Synthesis of Dimers



Lit refs: D.K. Donald et.al. Tetrahedron Letters p1375, 1991, P.Kocovsky, Tetrahedron Letters p5521, 1992

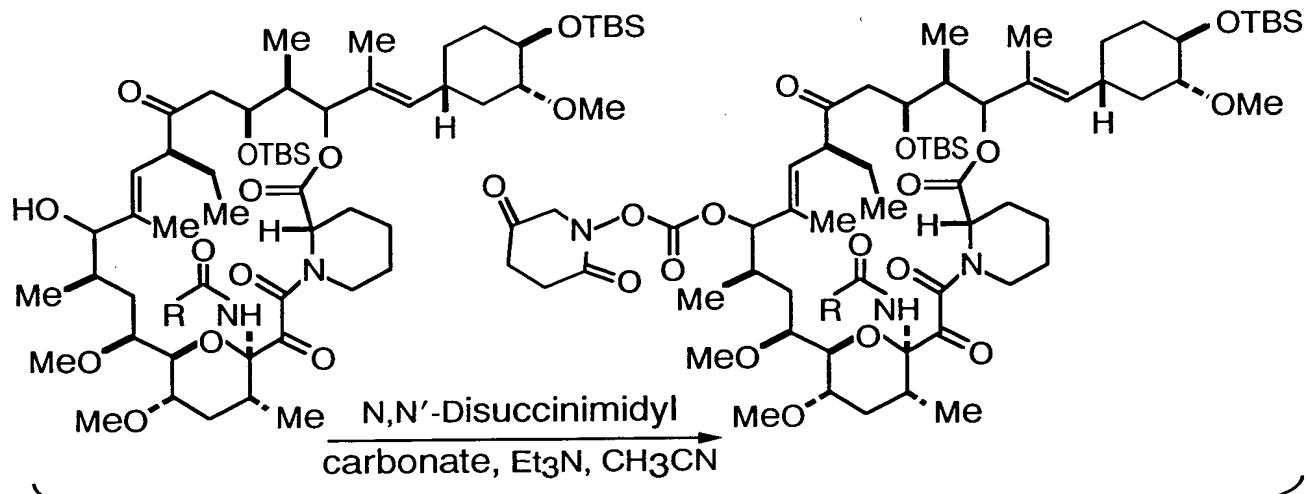


FIG. 11A

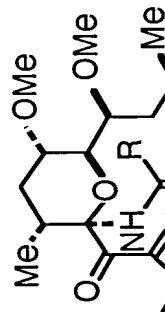
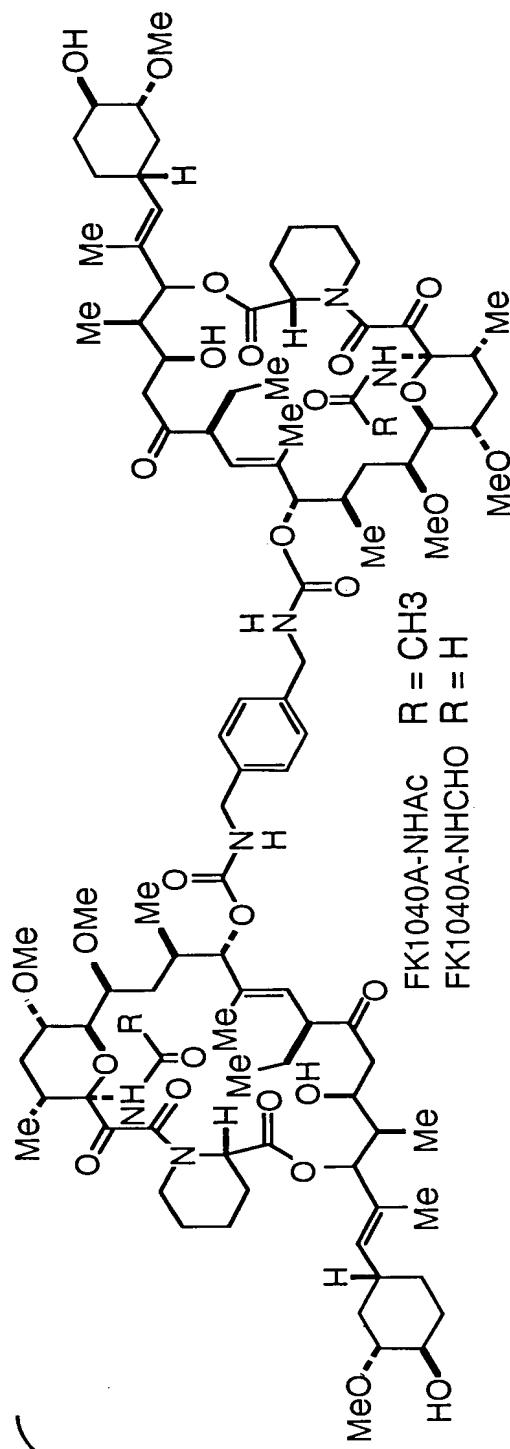


FIG. 11B

An additional modified FK520 (FK1040) that interferes with FKBP12 yet should bind the FKBP12 mutant: F36A or F99A or Y26A, or combinations thereof is

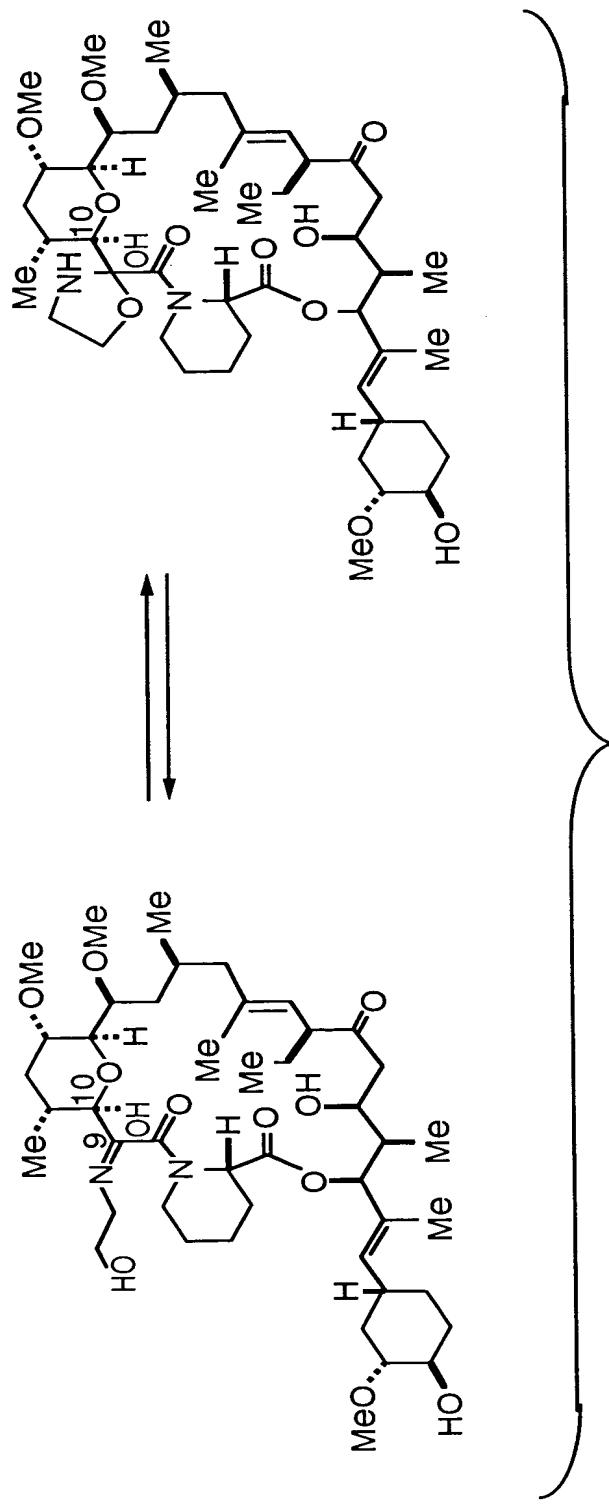


FIG. 11C

Scheme 3 Heterodimerization

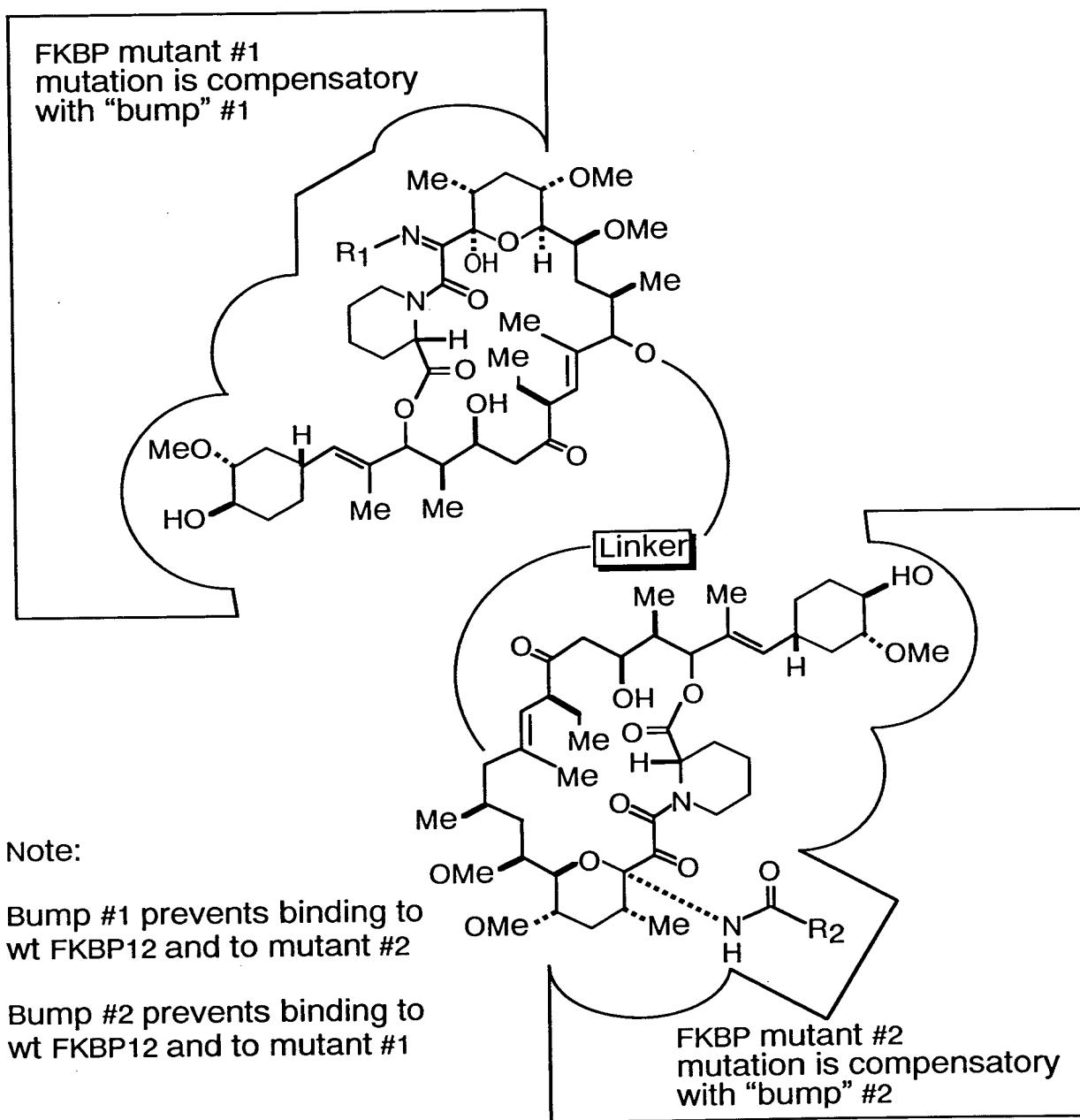


FIG. 12

Scheme 3: Synthesis of heterodimers

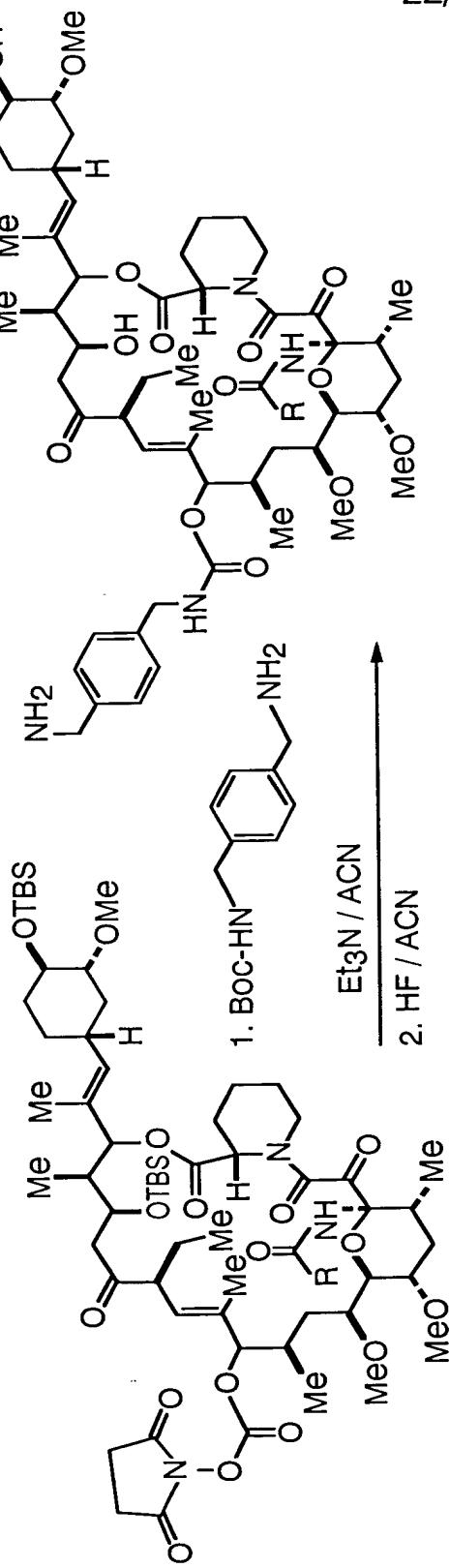
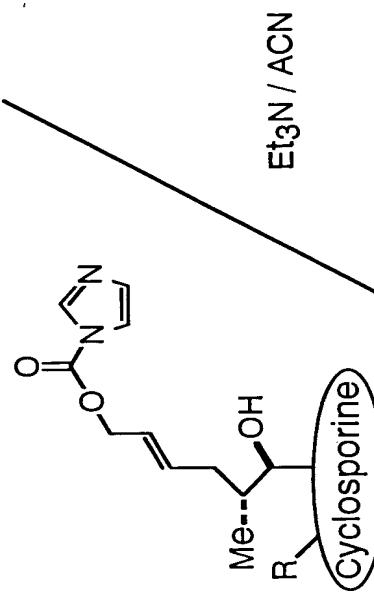


FIG. 13A



TO FIG. 13B

FROM FIG. 13A

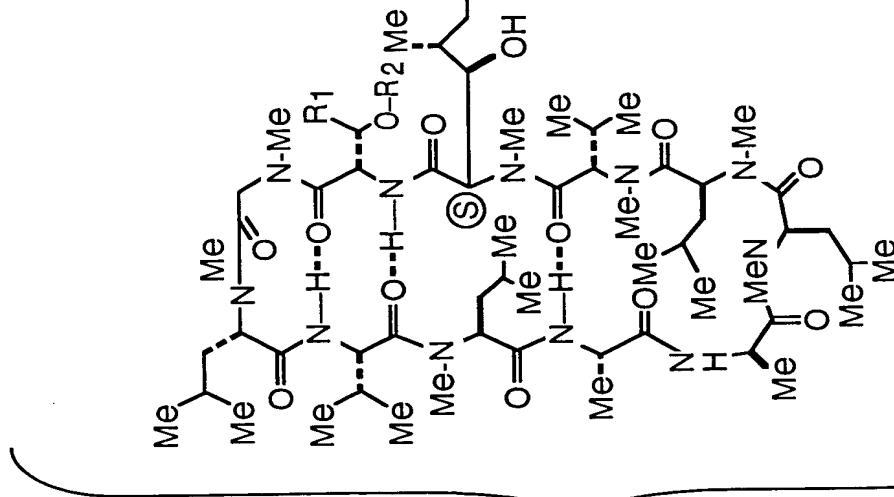
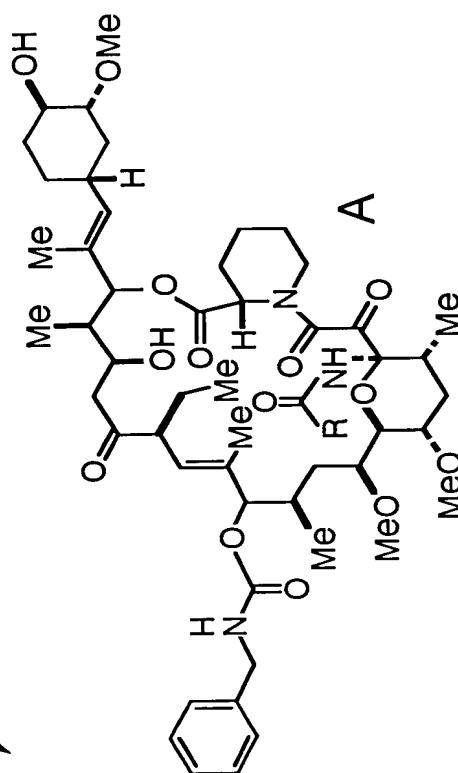


FIG. 13B

In this example, a heterodimer of a cyclosporine analog and FK520A-NHCO-R were heterodimerized. However, the scheme can easily incorporate other FK506/520 derivatives to form hetero or homodimers

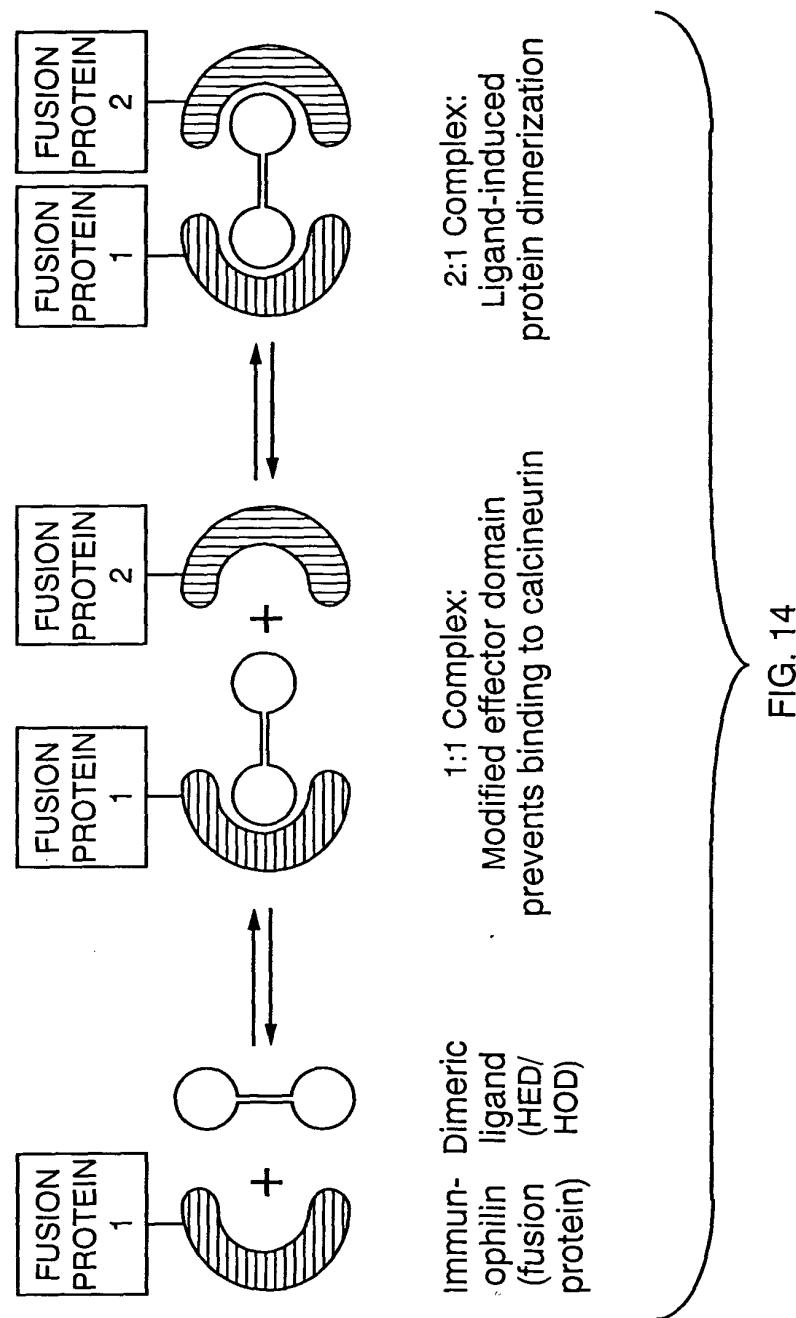


FIG. 14

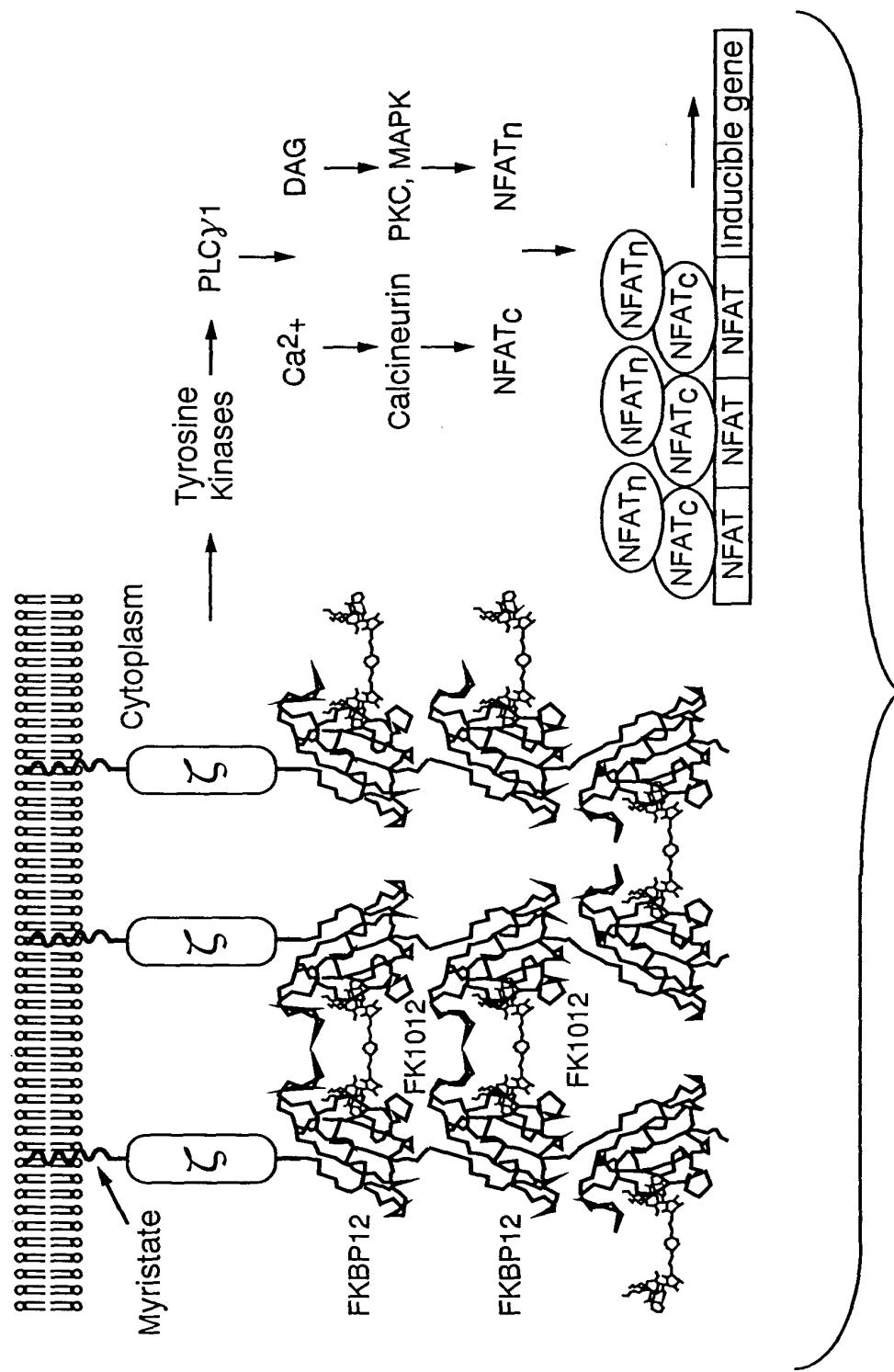
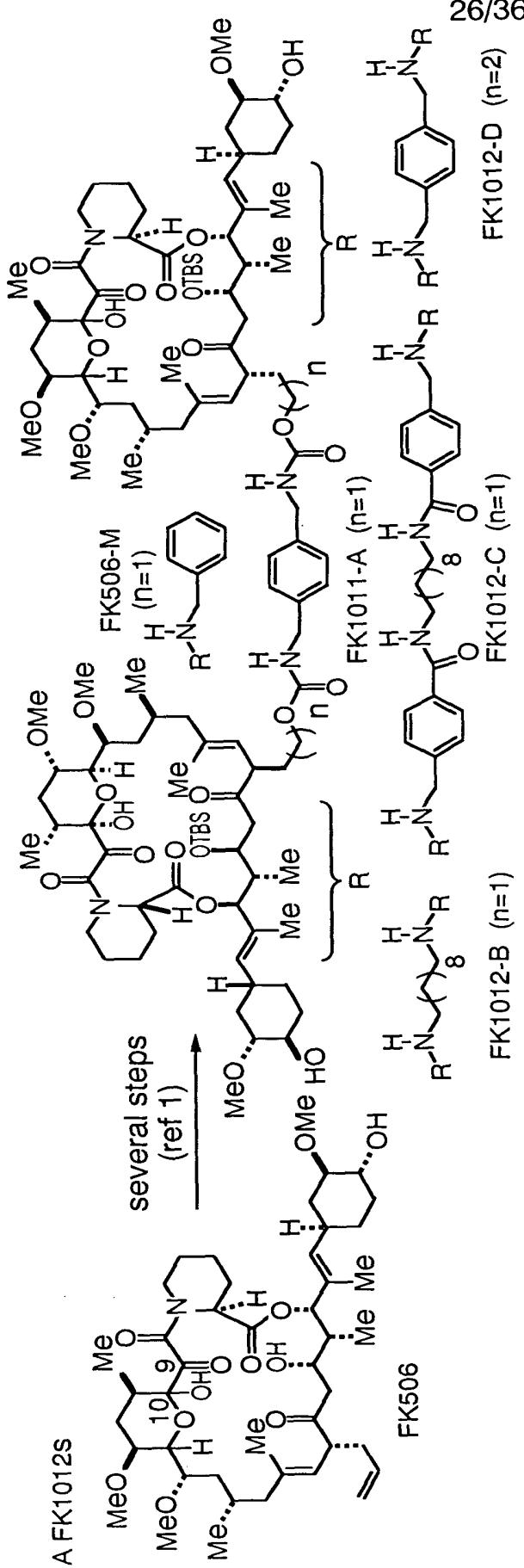


FIG. 15



B FK506 Monomer with a C10 Bump

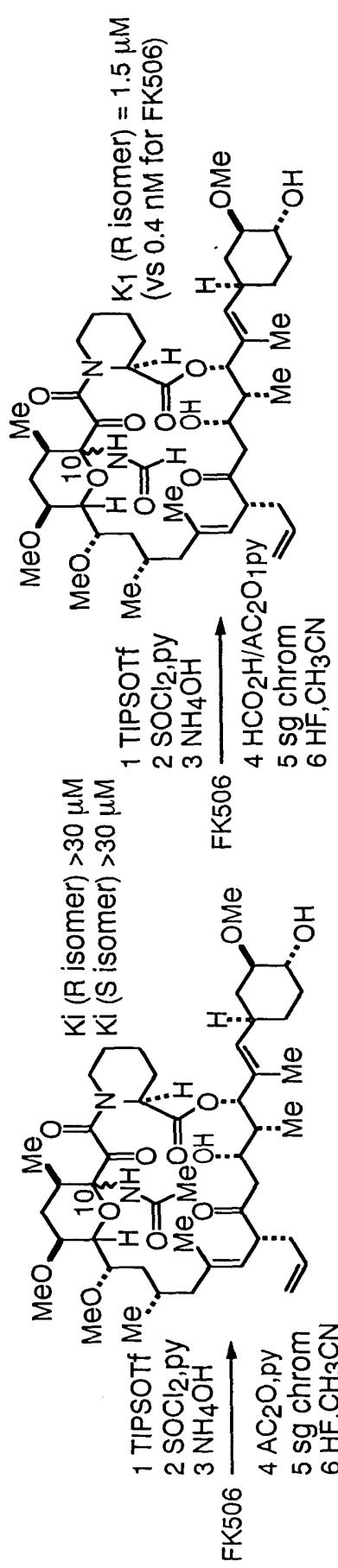
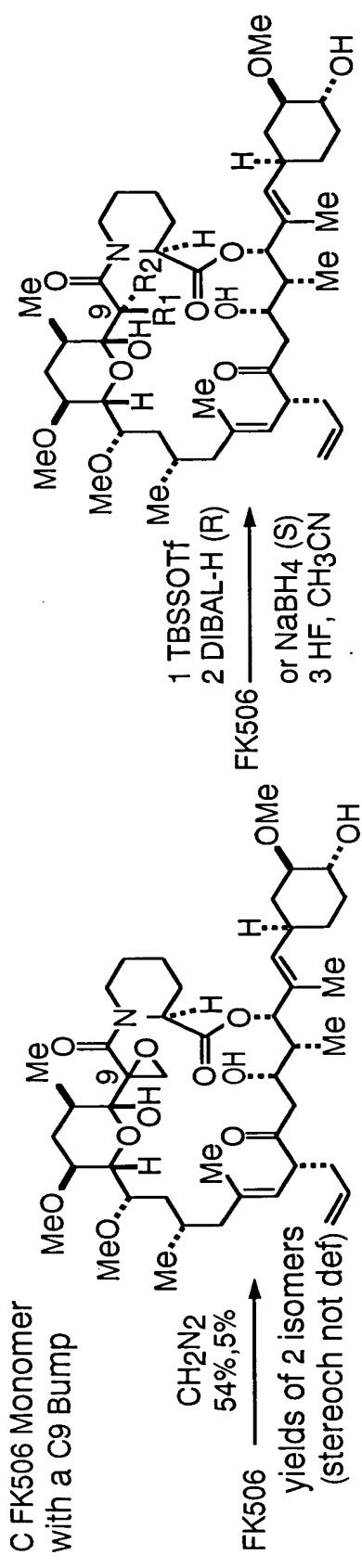
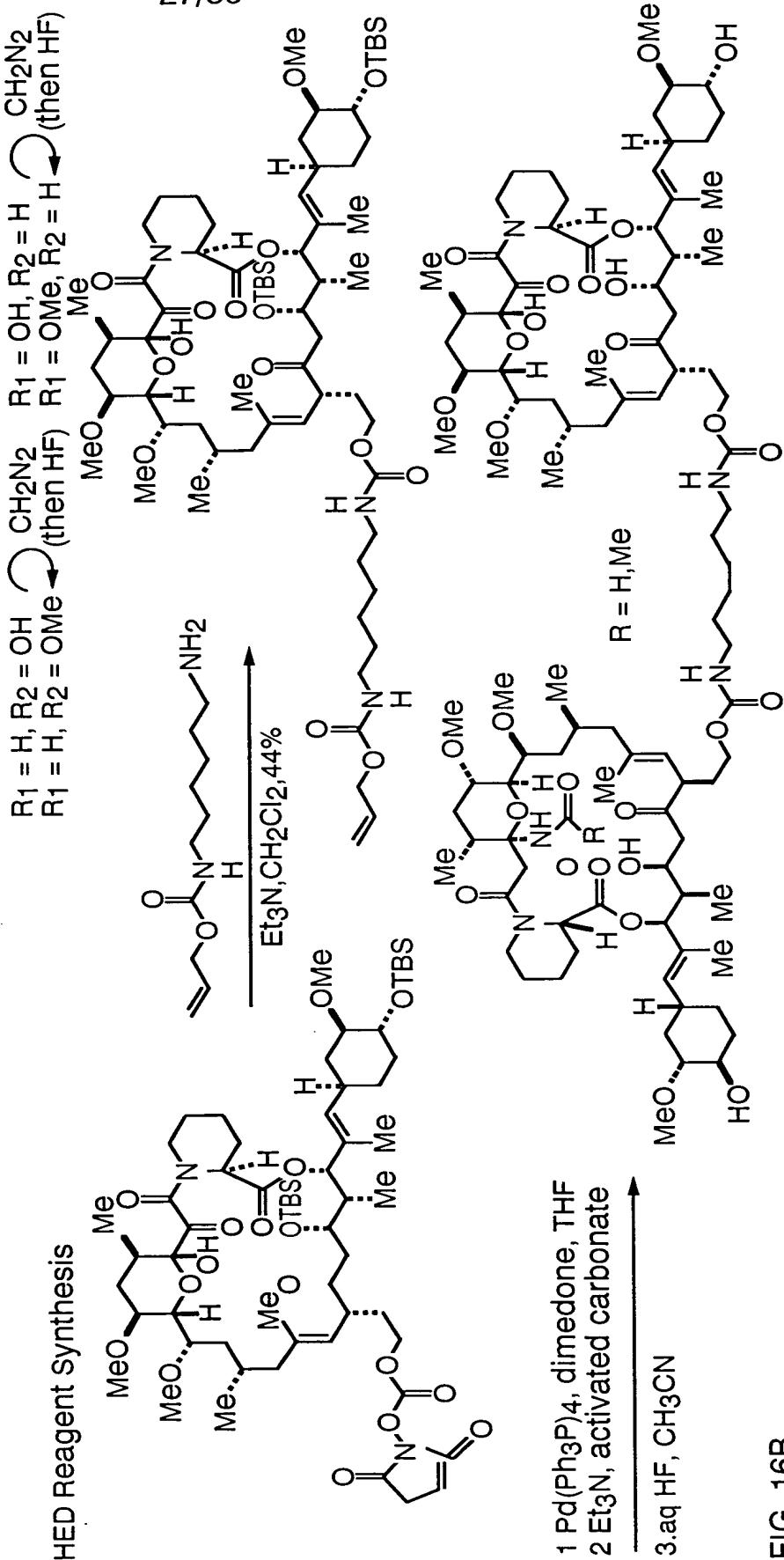


FIG. 16A



D HED Reagent Synthesis



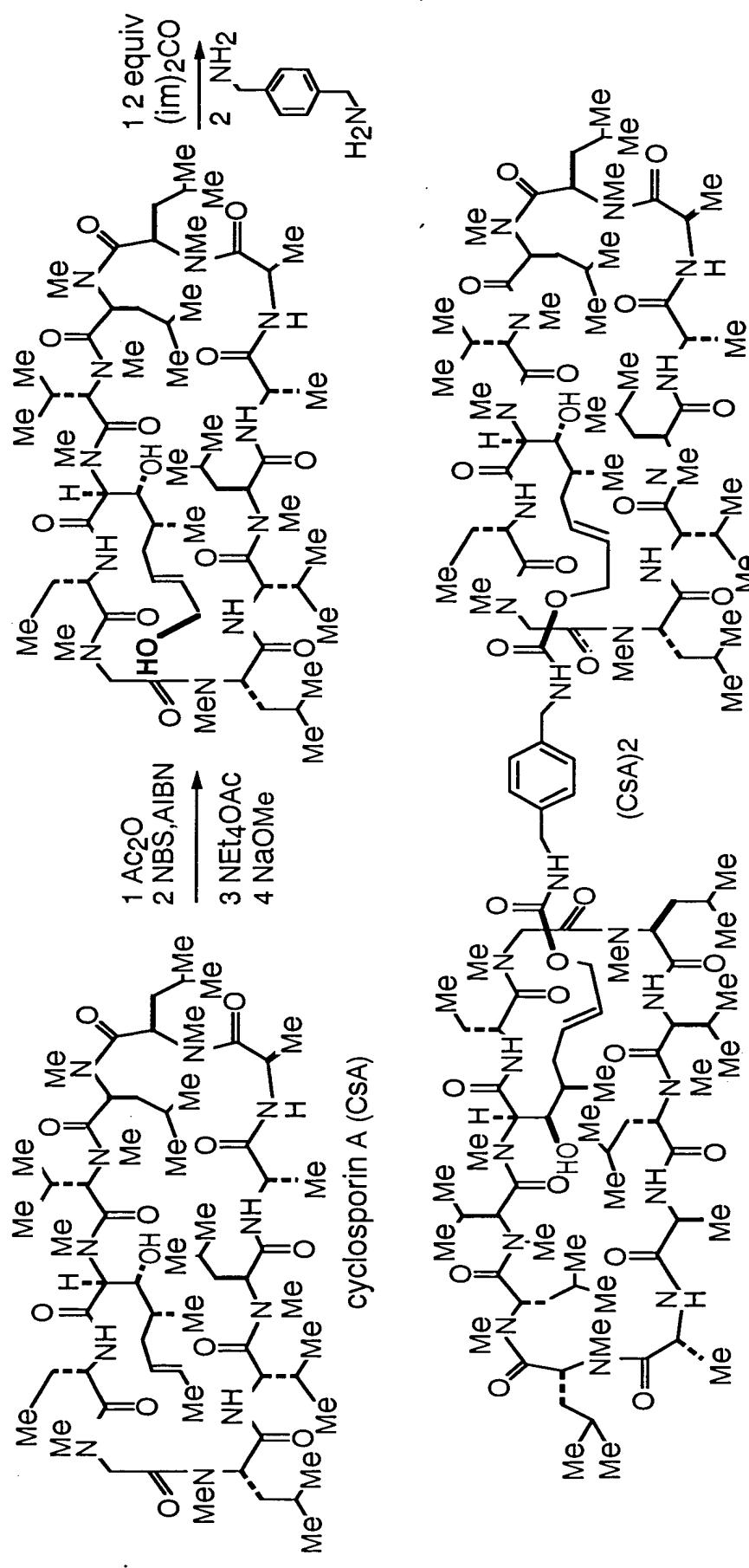
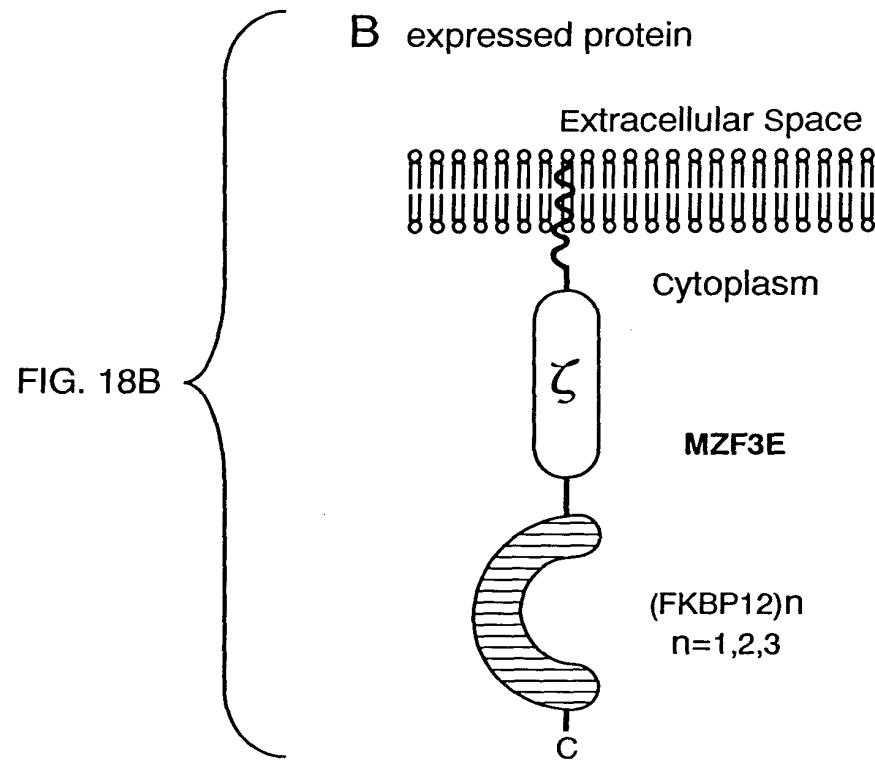
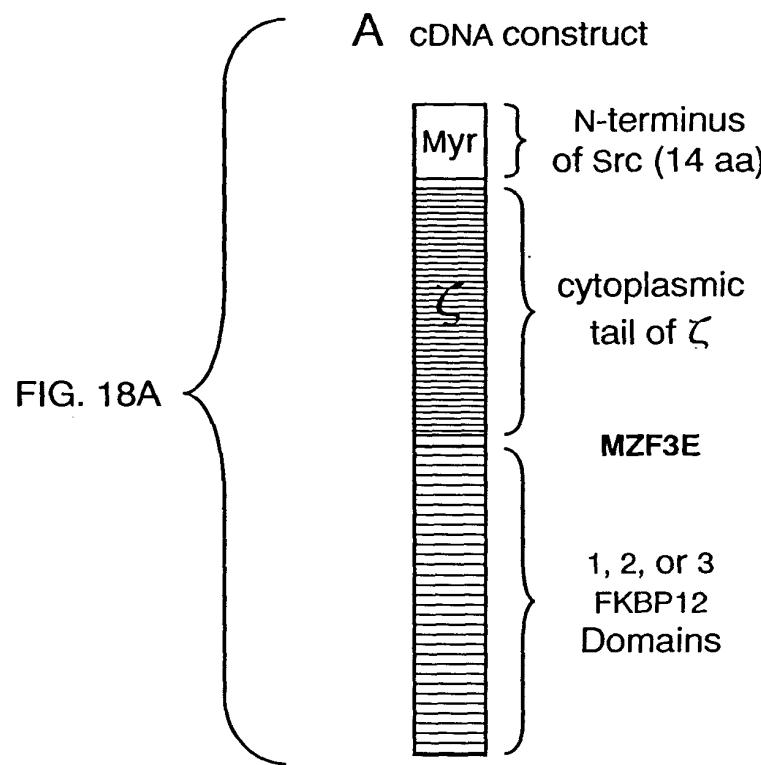


FIG. 17



1

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FK1012

— +

1

MZF1E<sub>f,h</sub> -  
(28kDa)

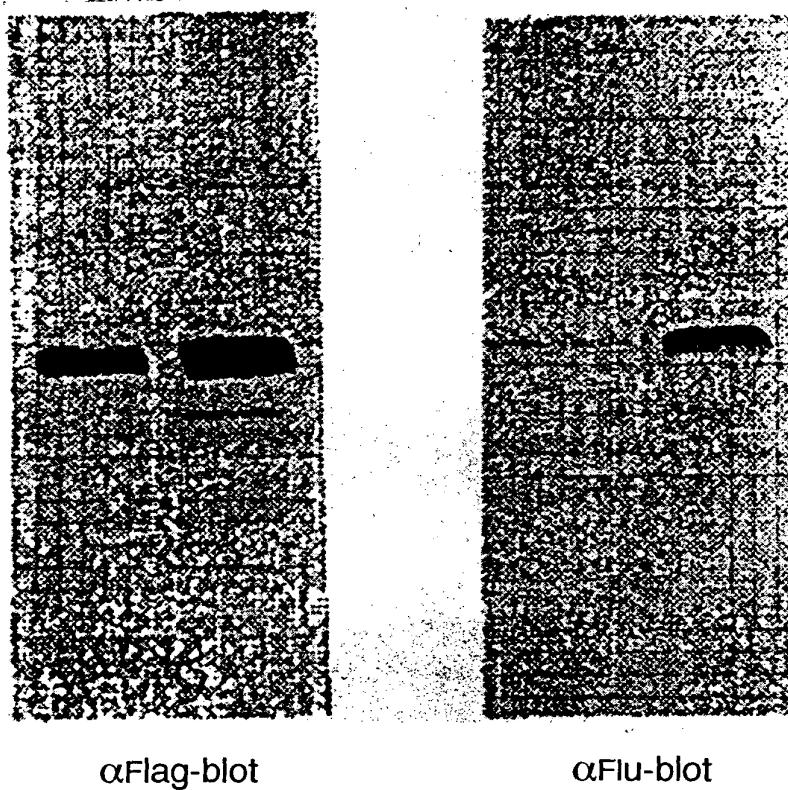


FIG. 19

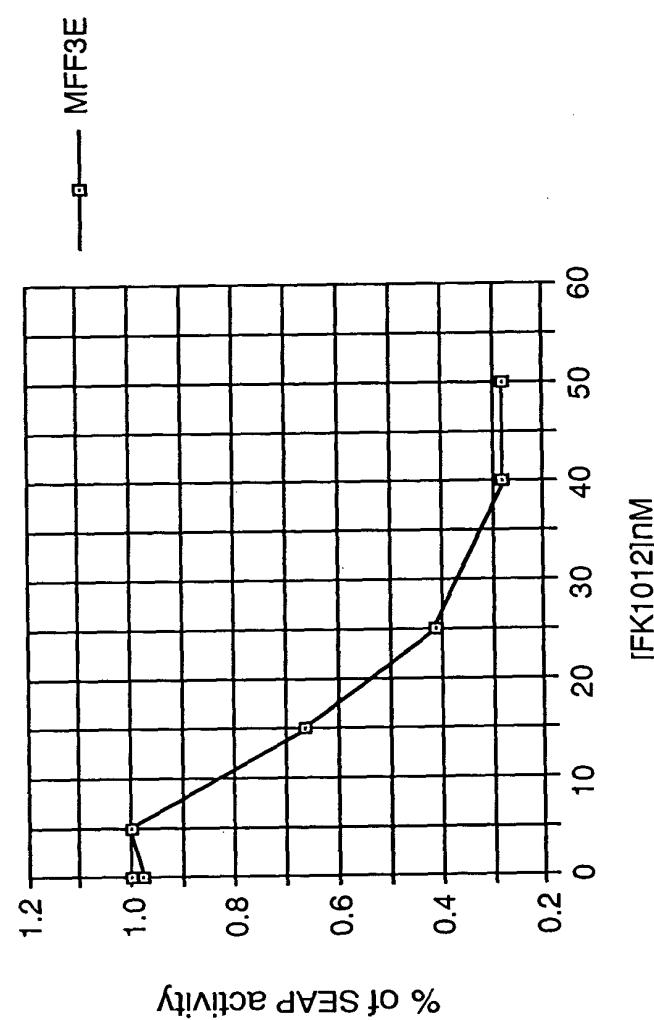


FIG. 20

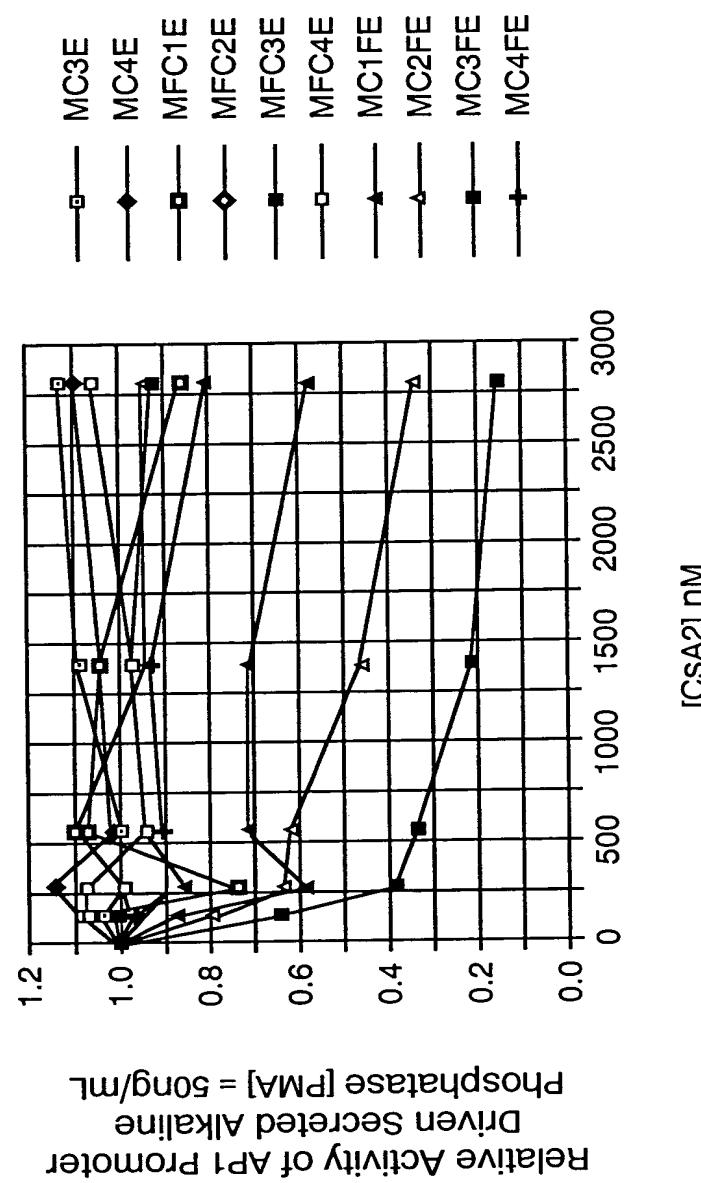


FIG. 21A

	LD50 Jurkat Cells Protein Expression				15nM	+		
MFF3E	Myr	Fas	FKBP	FKBP	Ep			
A								
MFC1E	Myr	Fas	CypC	Ep		NA		
MFC2E	Myr	Fas	CypC	CypC	Ep	NA		
MFC3E	Myr	Fas	CypC	CypC	CypC	NA		
MFC4E	Myr	Fas	CypC	CypC	CypC	NA		
MC1FE	Myr	CypC	CypC	Fas	Ep	500nM		
MC2FE	Myr	CypC	CypC	CypC	Fas	Ep	300nM	
MC3FE	Myr	CypC	CypC	CypC	Fas	Ep	200nM	
MC4FE	Myr	CypC	CypC	CypC	CypC	Fas	Ep	NA
MC3E	Myr	CypC	CypC	CypC	CypC	Ep	+/-	
MC4E	Myr	CypC	CypC	CypC	CypC	Ep	+++	
							>30 uM	
							>30 uM	

FIG. 21B

1

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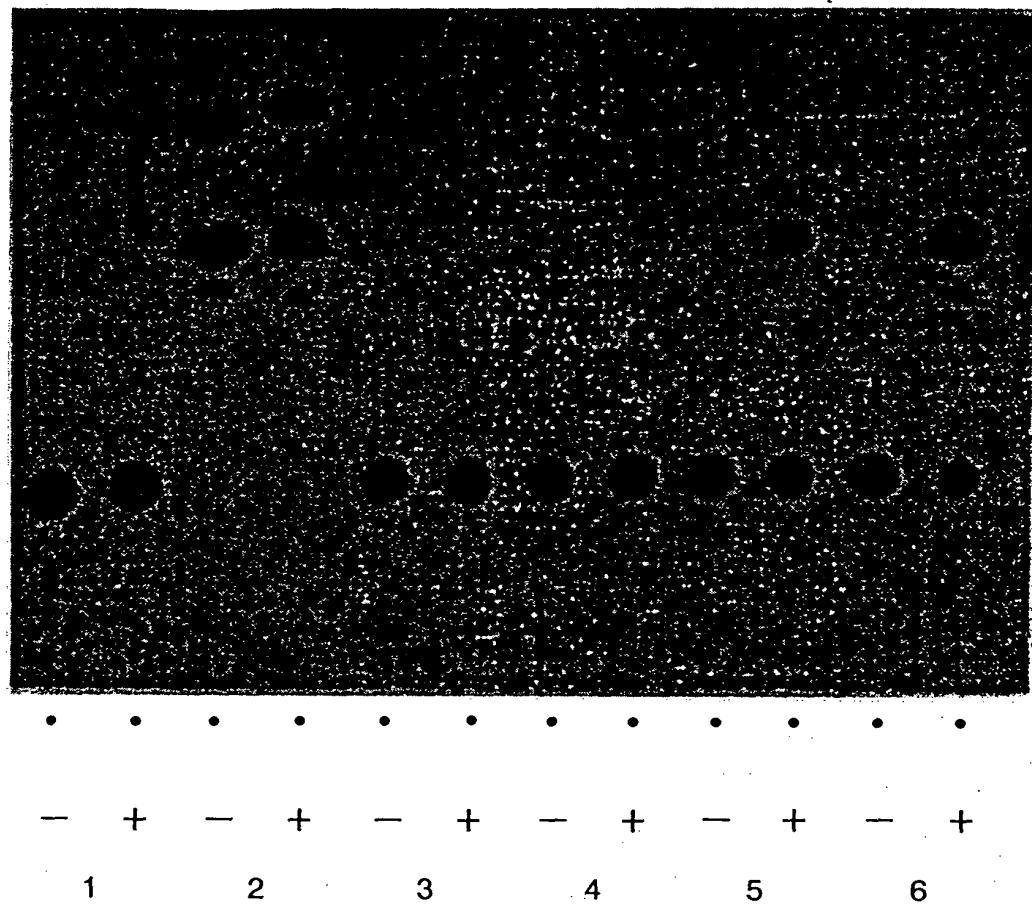


FIG. 22

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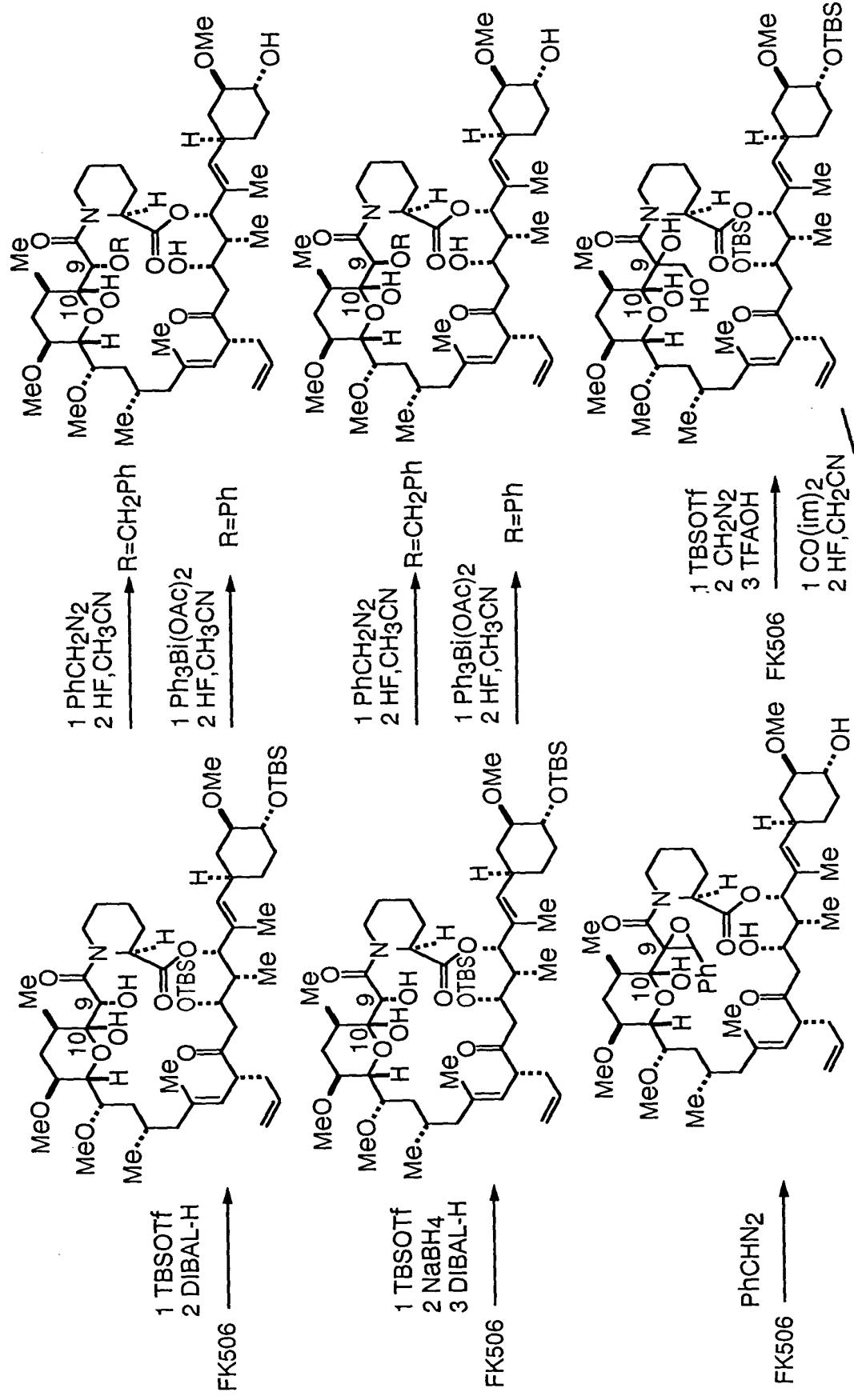


FIG. 23A

TO FIG. 23B

